

# **‘Breast Cancer Related Lymphedema’**

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## Abbreviations

ALND	=	Axillary Lymph Node Dissection
APTA	=	American Physical Therapy Association
BCRL	=	Breast Cancer Related Lymphedema (of the arm)
BCT	=	Breast Conserving Therapy
(SF-)BIA	=	(Single Frequency) BioImpedance Analysis
BIS	=	BioImpedance Spectrometry
BLE	=	Breast Lymphedema
BMI	=	Body Mass Index
ChTx	=	Chemotherapy
CT	=	Computed Tomography
CTCAE	=	Common Terminology Criteria for Adverse Events
DASH	=	Disability of the Arm, Shoulder and Hand scale
DEXA	=	Dual Energy X-ray Absorptiometry
EORTC	=	European Organization for Research and Treatment of Cancer
FACT-B	=	Functional Assessment of Cancer Therapy – Breast
HTx	=	Hormone Therapy
IOC	=	Impact Of Cancer scale
IPC	=	Intermittent Pneumatic Compression
LBCQ	=	Lymphedema and Breast Cancer Questionnaire
LMQ	=	Lower Medial Quadrant
LLQ	=	Lower Lateral Quadrant
MLD	=	Manual Lymph Drainage
MMD	=	Moisture Meter D device
MRI	=	Magnetic Resonance Imaging
NFBSI	=	National Comprehensive Cancer Network Assessment of Cancer Therapy - Breast Cancer Symptom Index
QLQ	=	Quality of Life Questionnaire
R	=	Resistance
RTx	=	Radiotherapy
SLD	=	Self Lymph Drainage
SNB	=	Sentinel Node Biopsy
TDC	=	Tissue Dielectric Constant
TWC	=	Tissue Water Content

ULQ = Upper Lateral Quadrant

UMQ = Upper Medial Quadrant

## **Abstract**

Improvements in the treatment of breast cancer have resulted in better survival rates and less breast cancer related morbidity. Nevertheless, a significant group of patients still experience a diminished quality of life as a result of lymphedema. In the early, often reversible, stage of lymphedema patients can experience subjective changes in the affected area. However, with the traditionally available tools the lymphedema often remains clinically undetectable and patients are denied essential care that can prevent worsening. Furthermore, most lymphedema assessment tools fail to support a clear unambiguous definition of lymphedema. This underlines the need for a sensitive objective measurement method that can assess lymphedema in a subclinical stage.

In this study we demonstrated that measuring tissue dielectric constant (TDC) using the MoistureMeter-D is an effective method to detect tissue water changes and could potentially provide a cost-effective adequate tool to measure the early onset of breast cancer related lymphedema (BCRL). Secondly, we established the correlation between the novel TDC method and the frequently used arm volume measurements and self-assessment questionnaires.

A group of 20 female patients with clinically BCRL were included. TDC measurements in both arms and all quadrant of both breast were recorded along with volumetric measurements of both arms. All patients were asked to complete a self-report questionnaire.

The novel TDC method detected significantly higher tissue water levels in the affected arm and breast compared to the control side. The TDC ratio between control and affected side showed significant correlation with self-reported pain and discomfort in both arm and breast. In the arm, the TDC method also showed correlation with the volume measurement method. The TDC value of the arm was correlated to age, but not to BMI.

This study demonstrates that measuring TDC using the MMD is an effective method for quantifying lymphedema in arm and breast and is an important tool in detecting early TWC changes.

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## Table of content

<b>1.0 Introduction</b>	<b>9</b>
1.1 The lymphatic system	10
1.2 Pathophysiology of lymphedema	13
1.3 Primary vs. Secondary lymphedema	14
1.4 Clinical Presentation	15
1.5 Breast Cancer Related Lymphedema (BCRL) of the arm	17
1.6 Breast Lymphedema (BLE)	18
1.7 High risk breast cancer patients	19
1.8 Diagnosis of breast cancer related lymphedema	20
1.8.1 Self-Report	21
1.8.2 Circumference and volume measurements	22
1.8.3 Ultrasound	25
1.8.4 Magnetic resonance imaging	26
1.8.5 Lymphoscintigraphy	27
1.8.6 Bioimpedance spectrometry (BIS)	30
1.8.7 Computed Tomography	33
1.8.8 Tonometry	33
1.8.9 Dual energy X-ray absorptiometry (DEXA)	34
1.8.10 Tissue dielectric constant	36
1.9 Therapy	39
1.9.1 Compression Therapy	40
1.9.2 Manual Lymph Drainage (MLD)	42
1.9.3 Physiotherapy, exercise and elevation	44
1.9.4 Pharmaceutical therapy	46
1.9.5 Surgical treatment	48
1.9.6 Heat therapy	50
1.9.7 Low level laser therapy	51
1.9.8 Skin care	52
<b>2.0 Aims of this study</b>	<b>53</b>
2.1 Aims of this study	54

<b>3.0 Methods</b> .....	<b>56</b>
3.1 Patients .....	57
3.2 Volume measurements .....	57
3.3 Tissue dielectric constant (TDC) measurements .....	59
3.4 Tissue dielectric constant model of the skin .....	61
3.5 Self-reported measures of lymphedema .....	67
3.6 Data analysis .....	67
<b>4.0 Results Arm lymphedema measurements</b> .....	<b>68</b>
4.1 Patients .....	69
4.2 Self-reported measures of lymphedema .....	69
4.3 TDC measurement of the arm .....	71
4.4 Volume measurements .....	72
4.5 Comparison of arm TDC, volumetric and self-reported measures .....	74
<b>5.0 Results Breast lymphedema measurements</b> .....	<b>76</b>
5.1 Patients .....	77
5.2 Self-reported lymphedema .....	77
5.3 Breast TDC measurements .....	79
5.3.1 TDC measurements of the controls breasts .....	82
5.3.2 TDC measurements of the post-mastectomy breast .....	82
5.3.3 TDC measurements of the post-‘breast conserving therapy’ breast .....	83
5.4 Comparison of breast TDC and self-reported measures .....	83
<b>6.0 Discussion</b> .....	<b>87</b>
6.1 Background and method consideration .....	88
6.1.1 Volume measurement of the arm and TDC .....	90
6.1.2 Localization of arm TDC measurement .....	91
6.1.3 Arm TDC and age .....	92
6.1.4 Arm TDC and BMI .....	94
6.1.5 Arm TDC related to surgery and self-reported measures .....	95

6.2 Breast lymphedema .....	96
6.2.1 Localization of measurement.....	96
6.2.2 Breast skin TDC and probe depth .....	98
6.2.3 Breast TDC correlated to age and BMI .....	100
6.2.4 Breast TDC related to surgery type and self-reported measures .....	100
6.3 Clinical cut-off point for arm and breast lymphedema .....	101
6.4 Future research .....	102
6.5 Conclusion.....	103
<b>References .....</b>	<b>104</b>
<b>List of publications .....</b>	<b>117</b>
<b>Appendix 1 .....</b>	<b>119</b>
<b>Appendix 2 .....</b>	<b>122</b>
<b>Appendix 3 .....</b>	<b>126</b>
<b>Appendix 4 .....</b>	<b>128</b>
<b>Appendix 5 .....</b>	<b>132</b>
<b>Appendix 6 .....</b>	<b>136</b>

# 1.0 Introduction

## 1.1 The Lymphatic system

The lymphatic system of the human body consists of several organs that can be divided into central or primary lymphoid organs (thymus and bone marrow) where lymphoid precursor cells undergo antigen independent proliferation and differentiation, and the peripheral or secondary lymphoid organs (including lymph nodes, spleen, Peyer's patches in the lamina propria of the ileum, lymphoid nodules of gastrointestinal and other tracts) where functional lymphocytes go.

Besides the important role of the lymphatic vessels in the immune system, the lymphatic vessels also regulate the maintenance of the homeostasis of the interstitial physiology. The interstitium may be defined as the space located between the capillary walls and the surrounding cells. The fluid in the interstitium contains a combination of nutrients and waste product of the tissue such as: amino acids, sugars, fatty acids, coenzymes, hormones, neurotransmitters, salts, urea and white blood cells. Exact composition differs slightly depending on the histology of the tissue. The amount of interstitium varies from about 50% of wet weight in skin to 10% in skeletal muscle. The normal function of the lymphatic vessels is drainage of a minor part of the interstitial fluid in to the venous system.

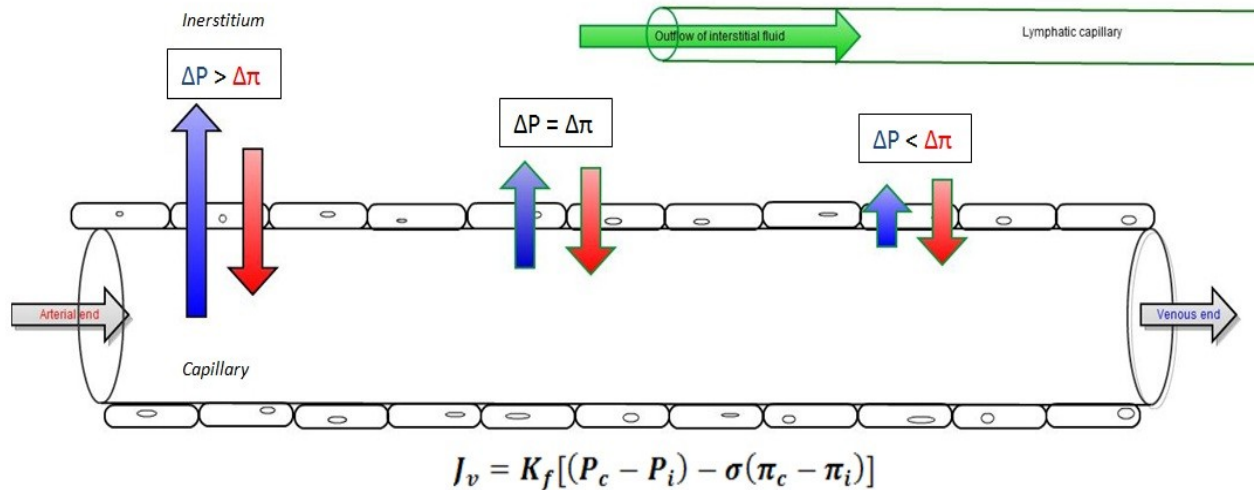
After the nutrient and oxygen rich blood reaches the arterial capillaries the hydrostatic pressure generated by the systolic force of the heart pushes water and small solutes out of the capillaries into the interstitial space adding to the interstitial fluid. Fluid exchange between capillaries and interstitium is a result of differences between the hydrostatic and plasma colloidal osmotic pressure inside the capillary vessel and the hydrostatic and the interstitial fluid colloidal osmotic pressure in the interstitium[1]. The general dynamics of this flow is captured by the 'Starling equation':

$$J_v = K_f[(P_c - P_i) - \sigma(\pi_c - \pi_i)]$$

In which the driving pressure across the capillary ( $J_v$ ) is determined by the difference in hydrostatic pressure ( $P_c - P_i$ ) and the difference in colloid osmotic pressure ( $\pi_c - \pi_i$ ).  $K_f$  is the filtration coefficient which is the product of two components: the capillary surface area and the permeability of the

capillary surface. The reflection coefficient,  $\sigma$ , is a measure of the selectivity of the membrane to a particular solute, and thus depends on both the properties of the membrane and solute. When the molecules can transverse freely over the membrane there's no osmotic pressure and thus  $\sigma=0$ . A reflection coefficient of 1 means a completely impermeable membrane with 100% reflection of the solutes. In practice  $\sigma < 1$  because solutes may penetrate the membrane and thus only a fraction the full osmotic pressure is noticed. Since the majority of the osmotic pressure is generated by large molecule plasma proteins (70-80% of all plasma protein is albumin, a lesser contribution comes from globulins) and the reflection coefficient of smaller molecules is close to zero, the reflection coefficient in the 'Starling' equation is often based upon the permeability of albumin.

On the arterial side of the capillary a positive hydrostatic pressure inside the vessel and the lower hydrostatic pressure outside the vessel force water out of the micro vessel. A higher colloid osmotic pressure inside the vessel creates a force in the opposite direction. Since the positive hydrostatic force at the arterial end of the capillary transcends the negative colloid osmotic pressure the net driving force result in a rapid outflow of fluid into the interstitium. On the way to the efferent venous side of the capillary the hydrostatic pressure inside the vessel drops significantly and the colloid osmotic pressure in the interstitium increases slightly while the rest of the components in the equation remain the same. The positive hydrostatic pressure inside the vessel no longer exceeds the negative colloid osmotic pressure resulting in a reverse flow and reabsorption of fluid from the interstitium (*see Fig. 1*). About 90% of all the fluid that has filtered out is reabsorbed this way. The remaining 10% of fluid and plasma protein that can't pass the membrane is drained by the unidirectional flowing lymphatic vessels[2].



**Fig. 1:** Schematic interpretation of the fluid transport over the capillary membrane as described by the Starling equation.

The lymphatic system is an accessory route by which fluids can flow from the interstitial spaces into the blood stream. And, most importantly of all, the lymphatic vessels can carry proteins and large particles away from the tissue spaces, neither of which can be reabsorbed directly into the blood capillary. Lymph capillaries are irregularly shaped blind-ending vessels which are significantly larger than blood capillaries. The flow of excessive fluid from the interstitium into the lymph capillaries is induced by the sub-atmospheric pressure inside the lymph capillary. In these lymph capillaries endothelial cells do not form tight junctions allowing fluid and large particles to enter the lymphatic system. Lymph capillaries merge to form lymph collecting vessels. Flow in these relatively larger vessels is initiated by compression from surrounding tissues[3]. The direction of flow is determined by the valves in the vessels prohibiting the lymph fluid to flow back. The collecting vessels come together in a network of lymph nodes, where lymphocytes and macrophages filter out cell debris and infectious agents from the lymph circulation. The afferent lymph vessels lead away the filtered lymph fluid from the lymph node before finally congregating in lymph ducts. In contrast to the capillaries the lymph ducts (and some of the smaller vessels) contain a layer of smooth muscle cell in their wall. Flow within the lymph duct is partially generated by peristaltic contractions of the smooth muscle in addition to compression of the duct by surrounding tissues. As in the smaller lymph vessels the

direction in the lymph duct is also regulated by valves preventing back-flow. All lymph fluid of the upper right side of the body, including the right sides of the head, neck, and thorax and the entire right upper extremity end up in the right lymphatic duct that ends at the right subclavian vein at the junction of the right internal jugular vein. The thoracic lymph duct receives lymph from the rest of the body including both lower limbs. It starts off in the abdomen and runs upward through the thorax and enters the left subclavian vein at the junction of the left internal jugular vein. The lymphatic system is also essential in the uptake of particular nutrients. While most nutrients absorbed by the small intestine are passed on to the portal venous system into the liver for processing, the lymphatic circulation is partially responsible for removal of fats from the digestive system, in the form of an opaque substance called chyle, before returning the fats to the venous system via the thoracic duct.

## **1.2 Pathophysiology of lymphedema**

Edema is often described as a (palpable) swelling as a result of the increase of fluid in the interstitium, due to an imbalance between capillary filtration and lymph drainage regardless of the underlying cause. The increase of fluid in the interstitium is a result of either imbalance between the hydrostatic and colloid osmotic pressure over the membrane of the capillary or insufficient uptake by the lymphatic system. Changes in capillary hydraulic pressure (e.g. heart failure, nephritic syndrome, venous obstruction, drug-induced), capillary permeability (e.g. trauma, inflammation or sepsis, allergic reactions, acute respiratory distress syndrome, diabetes mellitus), increase in interstitial colloid osmotic pressure (e.g. hypothyroidism or malignant ascites) or a reduction in the plasma colloid pressure (e.g. hypo-albuminaemia) can all lead to clinical edema. Edema can also be induced by lymphatic obstruction (e.g. nodal enlargement due to malignancy, lymph node removal) or lymphatic insufficiency (e.g. damaged lymph vessel, leaking valves), since the fluid that is normally filtered by the lymphatic system is not returned to the systemic circulation. In this case of interstitial fluid retention, known as lymphedema, the arterial/venous capillary filtration rate is unaffected. It

usually occurs when lymphatic outflow has been reduced by 80% or more. In contrast to other forms of edema, the interstitial protein concentrations raise significantly resulting in the accumulation of additional water due to osmotic pressure. The accumulation of interstitial fluid leads to massive dilatation of the remaining lymphatic outflow tracts and valvular incompetence that causes reversal of flow from subcutaneous tissues into the dermal plexus. In a later stage the lymphatic walls undergo fibrosis, and fibrinoid thrombi accumulate within the lumen, obliterating much of the remaining lymph channels. Spontaneous lympho-venous shunts may form. Lymph nodes harden and shrink, losing their normal architecture. In this advanced stage of the disease, protein and fluid concentration in the interstitium may initiate a marked inflammatory reaction. Macrophage activity is increased, resulting in destruction of elastic fibres and production of fibrosclerotic tissue. Fibroblasts migrate into the interstitium and deposit collagen causing irreversible damage. Consequently, local immunologic surveillance is suppressed, and chronic infections, as well as malignant degeneration to lymphangiosarcoma, may occur.

### **1.3 Primary vs. Secondary lymphedema**

Primary lymphedema is used to describe patients who have an abnormality or dysfunction in their lymphatic system, whereas secondary lymphedema results from disruption or obstruction of a normal lymphatic system due to disease or iatrogenic processes. Primary lymphedema including insufficient number of lymphatic capillaries, lymphatic hypoplasia or hyperplasia and functional insufficiency or absence of lymphatic valves[4] is idiopathic of nature and considered congenital[5]. Some patients may have an impairment in the intrinsic contractility of the lymphangion (the segmentally contracting, functional vascular unit of the lymphatic circulation), however not all primary lymphedema patients show defects in the lymphatic system.

Primary lymphedema is often associated with genetic diseases with known mutations of the FLT4 gene (previously known as VEGFR3) in Milroy disease[6] and of the FOXC2 gene in lymphedema associated with distichiasis[7], resulting in functional abnormality of the lymphatic system.

Most congenital malformations typically present in childhood with the exception of lymphedema praecox which remains unnoticed until puberty and lymphedema tarda of which the onset is not until the age of 35[8]. Primary lymphedema is found in both sexes, but women are more often affected than men[8].

Worldwide the dominant cause of secondary lymphedema is infection with *Wuchereria bancrofti* (and less common: *Brugia malayi* and *Brugia Timor*) resulting in a disease called *filariasis*[9]. The *Wuchereria bancrofti* is a nematode worm spread by mosquitoes and enters the human body in the bloodstream after which it migrates to the peripheral lymphatic system causing obstruction[10]. In the western world nearly all cases of secondary lymphedema are related to malignancy or its therapy. Secondary lymphedema following oncological treatment is supposedly caused by disturbed drainage due to damage to the lymphatic system. The majority is a result of breast cancer (treatment)[9], but lymphedema following treatment for sarcoma, melanoma, gynaecologic cancer and genitourinary cancer must not be underestimated[11]. Other non malignant causes that can lead to scarring and damaging the lymphatic system are inflammatory in nature and include infection or autoimmune diseases such as rheumatoid arthritis, trauma and immobilisation.

#### **1.4 Clinical presentation**

The onset of secondary lymphedema is often insidious. It often develops slowly and is completely painless at first. Initially the swelling of the affected area subsides during rest, but aggravates towards the end of the day. Without a distinct underlying cause it may sometimes be hard to differentiate between lymphedema and edema as a result of venous insufficiency based solitarily upon clinical presentation and so an additional test may be desirable. Nevertheless, not all lymphedema progresses gradually: a swelling caused by lymphedema may also be suddenly provoked by local inflammation from causes such as infection or limb injury. Regardless, the underlying cause and time of onset, in the first stage of lymphedema is characterized by a pitting swelling and often a positive 'Stemmer'- sign. The 'Stemmer'-sign is the inability to pinch and lift the

skin fold at the base of the second toe or second finger of the affected limb. However, the distribution of the swelling may be limited to only the proximal or distal part of the limb.

Lymphedema may also predispose to recurrent skin infections. Although most lymphedema is often associated with swollen extremities, it also does occur in the trunk, especially in the irradiated breast.

Eventually patients with lymphedema may report a wide variety of complaints, including heaviness, the perception of increased size, a tight sensation of the skin, or decreased flexibility of the affected joint. Pain may not always be present, but most patients suffer from a continuous discomfort in the affected area.

In a later stage the swelling of the affected limb or region may no longer be described as soft and pitting, but progresses to a more indurated state as the lymphatic walls undergo fibrosis. In this phase the skin hardens and the swelling no longer diminishes with elevation. Patients are now more prone to infections and intense treatment is necessary to reverse the process. If unsuccessful the slowed lymphatic flow might induce more fibrosis and the peripheral lymphatic system will lose more of its original architecture resulting in even higher fluid concentrations in the interstitium[2].

In this advanced stage of the disease, protein and fluid concentration in the interstitium may initiate a marked inflammatory reaction resulting in increased macrophage activity which eventually leads to destruction of the elastic fibres. The stasis of the lymph fluid in the interstitium might also trigger microbial proliferation causing lymphangitis, which leads to further destruction of lymphatic vessels. The loss of elastic fibres and the deposit of collagen are irreversible. Clinically, the subcutaneous tissue becomes firmer as fibrosis continues and hyperkeratosis, with or without verrucous or vesicular skin lesions, appears. The lymphedema is no longer pitting at this stage. Lymphedema can lead to various skin deformations which should be examined adequately since cutaneous malignant tumors, such as lymphangiosarcoma, Kaposi sarcoma, or lymphoma can be a rare complication of the disease and can have a delay of 10 up to 30 years after the onset of lymphedema[12-15].

Furthermore, patients with lymphedema have been found to be more disabled, experience a poorer quality of life, and have more psychological problems including frustration, distress, depression and anxiety[16, 17]. In addition, women reporting swelling have reported significantly lower quality of life in multiple functional assessments[16].

### **1.5 Breast cancer related lymphedema (BCRL) of the arm**

Although every year an increased number of patients are diagnosed with breast cancer[18], the developments in the treatment of breast cancer have increased survival rates significantly over the past two decades. Improved chemotherapy treatments, the introduction of neo-adjuvant chemotherapy and better targeted radiotherapy created the opportunity to treat a larger number of women with breast conserving therapy (BCT) with no differences in 15-year survival[19]. The introduction of the sentinel node procedure (SND) reduces the need for a complete axillary lymph node dissection (ALND) in many cases without compromising recurrence rates. As a result of these techniques the risk of arm morbidity, particularly lymphedema, sensory loss, and shoulder abduction deficits is significantly less[20-24]. Notwithstanding the development of better treatment options however, there is still a large group of women treated for breast cancer who develop complications associated with lymphedema.

Breast cancer related lymphedema (BCRL) can severely diminish the quality of life resulting in pain, sensation of swelling or heaviness (38%)[25] of the arm, limitation in shoulder movement (29%)[26], skin alteration[27] and an increased risk of infection[28]. Furthermore BCRL can have detrimental effects on work (36%)[25] .

Risk factors associated with BCRL lymphedema are believed to be the extent of surgical treatment (i.e. breast conserving therapy (BCT) versus mastectomy)[29, 30], the number of lymph nodes removed[21, 30-36], radiotherapy[30, 37], chemotherapy[38], high BMI and infection or trauma to the affected arm[39, 40]. Furthermore, some research relying on self assessment of lymphedema also associated tumor stage and tumor metastasis in axillary lymph nodes [38]

Although BCRL is the major cause of secondary lymphedema in developed countries, published incidence rates for BCRL vary substantially with a range of 2–65%[25, 41] depending on type of breast cancer treatment, lymphedema measurement method and lymphedema classification used. Over the years several classification schemes has been developed to describe the severity of lymphedema: The International Society of Lymphology introduced a classification where the stage of the disease is determined by difference in firmness, pitting and volume after 24 hours of elevation of the affected arm (Stage I: reversible, Stage II: irreversible by elevation, Stage III: lymphostatic elephantiasis)[42]. The American Physical Therapy Association (APTA) uses girth differences between ipsi- and contralateral arm as an measurement to classify lymphedema (<3cm: mild, 3-5cm: moderate, >5cm: severe)[43]. The National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) system combines both response to limb elevation and the presence of functional impairment. Typically, three quarters of all BCRL shows within the first three years after surgery[44, 45], although the onset of BCRL up to 20 years after surgery has been reported[40]. In contrast to general population of breast cancer survivors the peak prevalence for patients treated with loco-regional radiotherapy following surgery is at 10 years after surgery[44]. Although most studies agree on the increasing prevalence in the first three years, percentages still vary widely depending on lymphedema assessment method.

### **1.6 Breast cancer related lymphedema of the breast (BLE)**

Interestingly enough most research is focused solitarily on changes of volume and sensation of the arm, very few report on lymphedema in the breast itself, although many breast cancer survivors suffer from pain, heaviness, (perceived) swelling or discomfort in the operated area. Breast morbidity following breast cancer treatment has only been investigated in a few studies and is believed to affect approximately 40% of all patients[46-48]. Compared to other side effects, breast lymphedema (BLE) is sometimes considered as a minor problem, but BLE can cause serious difficulties in the

imaging and management of (recurrent) breast cancer in the follow-up, not to speak of the effect BLE has on the quality of life[48]. One of the main reasons why incidence numbers differ significantly and why so little is known about BLE is because there has not been a simple objective method to measure changes in interstitial fluid in the skin of the breast. Some of the techniques used to quantify lymphedema in the arm can, in theory, be good alternatives to quantify BLE, but with the exception of ultrasound[46] none of these measurement methods have been investigated properly. A measurement method that does not require a control breast has the potential to improve the detection of (early stages) of BLE since it also can be used in patients treated for bilateral breast cancer.

### **1.7 High risk breast cancer patients**

Different aspects of breast cancer treatment have been highly associated with the risk of BCRL. Women treated with a mastectomy have an increased risk of developing BCRL over women treated with breast conservative therapy. Numbers of women suffering from lymphedema after mastectomy differ because of variation in additional treatment: Mastectomy in combination with Sentinel Node (SN) 3-23%[25, 32, 49], mastectomy with additional axillary lymph node dissection (ALND) and no radiotherapy 30-47%[25, 50, 51] versus mastectomy with regional radiotherapy 58 -65%[25, 50]. Numbers BCRL after breast conservative therapy (BCT) are significantly lower but do increase with the involvement of ALND [25, 41].

Sentinel lymph node sampling (SN) has replaced axillary lymph node dissection (ALND) as the standard method of axillary staging for women with early-stage breast cancer. When it was introduced, it was assumed that there would be less morbidity such as BCRL. This statement has been proven by several groups [21, 31-36]. BCRL is believed to emerge in 1.9-5.3% of all cases when a sentinel node procedure is performed. A larger discrepancy is found when comparing numbers of BRCL in patients treated with ALND: 5.3-34.8%. These results can be partially explained by the differences in assessment methods and lack of agreement on the cut-off point for lymphedema.

Remarkable results were reported by McLaughlin et al. [33, 34] when comparing BCRL incidence based on objective measurement method (2 point circumference) and self-report. They found a much higher rate of self reported BCRL after ALND compared to what they measured (27% vs 16%). In contrast fewer women reported BCRL after being treated with SN procedure (3%) than what the circumference measurements showed (5%), emphasizing the unreliability of self-assessment. Furthermore, in most reports little is known on the number of nodes removed, the level of ALND and the need for additional radiotherapy. The percentage of removed breast tissue, the extend of the (axillary) lymph node removal and the potential additional radiotherapy, especially at the axilla, all negatively influence the chances of BCRL free survival. Tumor size and grade as well as additional chemotherapy and hormone therapy were not found to be influencing factors [52-54]. Furthermore, some advocate that older age also increases the risk of lymphedema after breast cancer surgery, although this view is not supported by others[52]. In contrast to age, it is believed that a high BMI does increase the risk of BCRL [52, 53]

### **1.8 Diagnosis of breast cancer related lymphedema.**

One of the reasons for the wide range of incidence numbers on BCRL is the lack of a common consensus on diagnosing and classifying BCRL, mainly because there's no unambiguous objective measurement method used to assess BCRL. Currently most studies use relative subjective methods, such as clinical examination in combination with circumference measurements and self-report. Other, more accurate, methods such as MRI, CT, ultrasound and limb volume measurements (perometry and the water displacement method) are often expensive, time consuming and they require training to operate, which makes them relatively unsuitable for daily clinical practise. Two promising new diagnostic tools are bio-impedance spectrometry and the assessment of tissue dielectric constant.

Diagnosing lymphedema in the skin of the breast relies in clinical practice completely on subjective methods like clinical examination or self-reports. Whereas the assessment of arm lymphedema in the

clinic is supported by relative objective methods such as circumference measurements and limb volume measurements, there are limited techniques available to determine changes in the breast. More research therefore needs to be done before these methods can be used in the assessment of breast lymphedema.

### **1.8.1 Self-Report**

A high percentage of women treated for breast cancer suffer from complaints such as perception of increased size, pain, heaviness, skin alterations or feelings of discomfort in the arm and breast leading to a diminished quality of life. Although these sensations are clearly related to BCRL, it is not ideal to use them as a quantitative tool to assess the level of lymphedema in the arm or breast. Breast cancer related lymphedema incidence rates differ within the group of studies relying on self-report. The outcome is highly dependent on the questionnaire used, how the questions are presented and language skills of the patient. There are several validated questionnaires that are also commonly used in the assessment of BCRL. One of the most commonly used questionnaires is the 'Functional Assessment of Cancer Therapy Breast + 4'. The 'Functional Assessment of Cancer Therapy Breast+ 4' (FACT-B+ 4) survey[55] is a Likert- type point scale on five items, often overestimating lymphedema when compared to objective assessment method[56]. The 6-item Functional Assessment of Cancer Therapy-Breast Symptom Index (FBSI) is similar and highly correlated with the FACT-B + 4[57]. An extended version of the FBSI is the National Comprehensive Cancer Network Functional Assessment of Cancer Therapy-Breast Cancer Symptom Index-16 (NFBSI-16), which includes all eight items from the original FBSI and eight additional items from Functional Assessment of Chronic Illness Therapy measures.

The 'Lymphedema and Breast Cancer Questionnaire' (LBCQ-Part I) is developed and revised by Armer et al[58] and is a tool containing 19 questions to assess signs and symptoms of limb volume changes. Another questionnaire differentiating between 'mild' and 'severe' lymphedema based upon a patient's own interpretation of arm swelling was developed by Norman et al.[59]. The

relationship between these surveys and more objective assessment methods has not been adequately investigated.

A non lymphedema specific 78-item questionnaire on the musculoskeletal condition of the upper extremities : Disability of the Arm, Shoulder, and Hand [DASH] Scale[60, 61] is also used frequently, but similarly to the 'FACT-B+4' often overestimates lymphedema when compared to more objective measurement methods such as bioimpedance spectrometry and volume measurements[56].

Other more widespread used questionnaires to assess the overall quality of life include The Short Form-36 (SF-36)[62] and The Impact of Cancer scale (IOC)[63], the more frequently used EORTC (European Organization for Research and Treatment of Cancer)-QLQ-C30 and the breast specific module EORTC-QLQ-Br23[64, 65].

Most studies use a simple four point ordinal scale (none, mild, moderate, or severe) to assess symptoms as pain/discomfort, heaviness, perceived swelling in order to quantify lymphedema[66].

Nevertheless, only some papers explored the correlation between the sensation of discomfort, swelling and heaviness with a more objective method like girth measurement[34, 67-69]. McLaughlin et al. reported a significant discordance between the presence of measured and patient-perceived lymphedema. Especially patients with a higher BMI or patients who had endured an infection or an injury in the affected arm were more likely to overestimate self-reported lymphedema[34].

### **1.8.2 Circumference and volume measurements.**

A more objective way to diagnose lymphedema is the use of volume measurements. The total arm volume can be compared to the contralateral arm (most common) or to previous findings[70].

Comparison with the contralateral arm is favourable because temporal fluctuations in BMI do not affect the outcome. For a reliable assessment of lymphedema, multiple measurements over a period of time are desirable since BCRL is a dynamic process that is thought to fluctuate, especially in the early stages of the disease. The total arm volume can be indirectly estimated from multiple

circumference measurement of the arm or measured with more accurate methods such as water displacement or perometry. In practice, water displacement is still the golden standard of volumetry, but the derivative technique of circumference is more commonly used since it's more applicable in everyday clinical practice.

In most research, circumference measurements are taken bilaterally at ten sites along the arm at 4cm intervals, with site 1 positioned at 19cm from the base of the nail of the third finger. However, others state fewer circumference measurements are sufficient to calculate the total limb volume measuring every 10cm[71-74]. Some even suggest that two measurements at a given distance above and below the olecranon are enough to indicate the presence of BCRL, advocating that accuracy is not dependable on the measurement interval [31, 70, 71, 75].

In research where diagnosis is based upon circumference (without the calculation of volume) the patient is often considered lymphedema positive when there's an increase of 2.0cm of the affected arm over the contralateral arm[31, 70, 72, 76-78], but some use stricter (>1.5cm) or more liberal (>2.5cm) definitions[79, 80]. Others rely on an increase of a certain percentage of the volume compared to the other arm[37, 81] or pre-operative measurements[82]. The different definitions and assessment methods of BCRL using solitary circumference make outcomes hard to compare[81].

Notwithstanding the lack of agreement on how to measure, research shows that the arm circumference method has a very low inter-observer bias once the same method is used[83].

Furthermore, when reducing the amount of measurements the assumption is made that lymphedema spreads evenly over the complete arm, therefore volume calculation is believed to be a more accurate way to assess arm lymphedema[84].

Arm limb volume is most frequently calculated from the circumferential measurements as the sum of

segment volumes using the formula for a truncated cone:  $V = h \times \frac{(C1^2 + C1 \times C2 + C2^2)}{12\pi}$ .

Although the sum of truncated cone calculations from multiple segments is more accurate than a single truncated cone measurement they're both equally reliable and closely related[85].

A more accurate assessment of the total arm volume can be done using the water displacement technique which is often seen as the golden standard: the patient submerges her arm in a cylinder filled with water and the overflow of fluid is collected and measured. Although the water displacement technique is known to be a sensitive and accurate method in the laboratory setting, it is seldom used in clinical settings because it's cumbersome. Additionally, a major concern is the sensitivity of this method to provide data about localization of the lymphedema or the shape of the extremity. Furthermore, this procedure is not advisable in patients with BCRL associated wounds or skin lesions.

Another way to determine arm volume is optoelectronic perometry. The perometer consists of a movable frame positioned at 90 degrees above a horizontal base plate. This frame contains rows of infrared light emitters on two sides at right angles to each other, which project light toward rows of light sensors on opposite sides. When a limb is placed in the measuring frame, it blocks the transmission of light creating a shadow. As the measuring frame is moved along the longitudinal axis of the limb, vertical and horizontal limb diameters are recorded every 5 mm and the total volume is calculated.

As with the circumference method it remains a subject of debate whether to determine the difference in volumes between the arms by absolute figures or percentages. Some consider the affected arm lymphedema positive when there's 200ml difference with the contralateral arm[74, 86, 87], or a 200ml difference with the preoperative measurement [88], others when there's an increase of 20% compared to the control arm[89] or 10% compared to preoperative assessments[82, 90].

Comparisons within the affected arm over time may overstate the incidence of lymphedema if total limb volume increase due to a build-up of adipose tissue. However, the results drawn from comparison between affected and unaffected arm may be falsified by pre-existing asymmetries that are not related to lymphedema. Therefore preoperative baseline measurements of both arms are preferable. In the general population the dominant arm frequently contains significant more volume

than the non-dominant arm[91]. If preoperative asymmetries are present adjustments should be made when quantifying lymphedema.

Unfortunately, the sensitivity of these assessment methods require that there must be enough lymphedema present to cause a detectable volume change, and is therefore unsuitable to diagnose lymphedema in an early stage.

### **1.8.3 Ultrasound**

Ultrasonography has an established diagnostic role in many fields of medicine. The main advantages of this non-invasive method of imaging are safety, high patient tolerability and relatively low cost. The technique of ultrasonography is based upon the detection of reflected sound waves through tissues that possess different acoustic properties. In general the resolution of the ultrasound images depends on the frequency used: the higher the frequency, the better the resolution. The geometric shape of the ultrasound beam also influences the resolution in the lateral direction. To assess changes of the skin most use 20 MHz high-resolution ultrasound imaging equipment [92-94], but some 10 MHz[95] transducers are also used. The widely available 7.5- and 10-MHz transducers are adequate for the examination of subcutaneous tissues, but the traditional ultrasound devices are not accurate enough to explore the skin.

Studies have shown that dermal echogenicity is inversely proportional to its concentration in water. Lymphedema results therefore in a loss of echogenicity of the skin in high-resolution cutaneous ultrasonography[93]. In normal skin, the dermis is echogenic and the subcutis has a hypoechogenic basal structure with diffuse hyper-echoic branches caused by connective tissue separating adipose lobules. In lymphedematous skin uniform homogenous hypo-echoic appearances are seen in the dermis when compared with the unaffected skin. Homogeneous dermal hypo-echogenicity confirms the particular appearance of lymphedema and that contrasts to the fluid retention localized only in the superficial dermis in venous insufficiency[94]. The water molecules in lymphedematous skin and subcutis found remain trapped by the high protein concentration at the point where it was formed.

In venous insufficiency the interstitial fluid is freer to move and often accumulates in the superficial dermis which is less dense and more vascularised than the deeper dermis. In patients diagnosed with lymphedema the dermal and subcutis thickness is often greater in the affected arm compared with the unaffected arm[95].

Although ultrasonography is harmless to the patient, easy accessible and cheap (around 120 euro per test), interpretation of changes based on echogenicity is not always evident. Furthermore, operating the ultrasound device and interpretation of the images requires training and more research needs to be done before ultrasound can be implemented in the routine of breast cancer related lymphedema assessment.

#### **1.8.4 Magnetic resonance imaging**

Magnetic resonance imaging is not used routinely but can be a helpful tool in the assessment of lymphedema. It offers details of the lymphatic system including possible pathologic dermal lymphatic vessels as well as more proximal lymph nodes and obstructing masses without the use of radiation or added contrast material. Besides, MRI is highly sensitive in confirming the diagnosis of lymphedema. Classical signs of lymphedema on MRI are skin thickening and a 'honeycomb'-structure due to fibrosis in the subcutaneous tissue (especially in the later stages of the disease), without edema of the muscles[96]. MRI scans, particularly  $T_2$ -weighted with fat subtraction, work well for differentiating fat from water, with water appearing brighter and fat darker. Therefore MRI may help clarify that limb enlargement represents fat deposition rather than fluid accumulation. When looking at the skin on  $T_2$ -weighted MRI images the intensity of the signal is higher (=brighter) in the dermis on the edematous side compared with the control side suggesting a higher concentration of fluid. In the segmental sections an increase of subcutaneous tissues in affected limbs often represents the most important contribution to the increase of total limb volume. In a study comparing the water displacement technique to MRI, to diagnose breast cancer related lymphedema of the arm, all patients not only showed a significant increase of the subcutis but also diffuse hyper intense signals

through the subcutaneous layer which indicates presences of water[95]. Furthermore, because of the ability to visualize deeper tissues MRI is also recommended when malignancy is suspected. The cost of a MRI scan including the report of a radiologist is between €250,- and €400,- in medical centres with a dedicated MRI room (which can cost up to €370.000,- to build).

### **1.8.5 Lymphoscintigraphy**

Lymphoscintigraphy, also known as isotope or radionuclide lymphangiography, is a technique that's based upon the uptake of large particles by the lymphatic system. Where in the past (blue) dye was used to visualize the lymph drainage of the limbs, nowadays the dye is substituted by radioactive labelled macromolecules or colloids made visible by X-ray.

The labelled macromolecules or colloids are injected intradermally in the web space of the foot or hand (usually between the hallux or pollux and the adjacent digit). However, there are some who advocate that an injection of the radioactive labelled substance near the side of the edema results in better understanding of the lymph drainage. The downside of this approach is that more distal parts of the lymph system might not be visualized. Regardless of the location of injection, in theory all the particles will be taken up by the lymphatic system and via the lymphatic collector ducts eventually passed on to the venous system. Massage at the site of injection or exercise after the injection has been shown to enhance the uptake of the particles into the lymphatic system significantly[97].

The clearance of molecules from the interstitial space depends largely on the size of the particle: particles with a diameter  $< 5\text{nm}$  can pass through the capillary wall and end up directly in the venous system, particles with a diameter  $>100\text{ nm}$  cannot be removed from the interstitial space by either lymphatic nor venous systems. Macromolecules and other particles with the size of  $\pm 10 - \pm 80\text{nm}$  can only be removed from the interstitial space by the lymphatic system, making them ideal to examine the lymphatic drainage and possible abnormalities. The difference in uptake with the size and characteristics of the molecules therefore affects the appearance of the lymphoscintigram. Particles may be removed directly into the lymphatic system or through phagocytosis by macrophages, which

then enter the lymphatic vessels. The two commonly used radio-active labelled particle groups are: macromolecules and colloids. To make them visible on a lymphoscintigram all particles must be labelled with radioactive marker (i.e. technetium [ $^{99m}\text{Tc}$ ]). There is no internationally agreed standardised technique and different substances are used:  $^{99m}\text{Tc}$ -HSA (human serum albumin),  $^{99m}\text{Tc}$ -HIG (human immunoglobulin),  $^{99m}\text{Tc}$ -antimony sulphide colloid,  $^{99m}\text{Tc}$ -sulphur colloid,  $^{99m}\text{Tc}$ -albumin colloid[98]. Generally, macromolecules produce better results in imaging lymphatic vessels since they are cleared faster than colloids. However, colloids are better trapped than macromolecules and there is less risk that they will enter the blood stream through the capillaries making them more suitable to visualize lymph nodes[98]. Since  $^{99m}\text{Tc}$ -HSA (human serum albumin) is believed to resemble the nature of the subcutaneous interstitial space the closest it's most frequent used. The choice of tracer may also be affected by the intention of the lymphoscintigram. Colloid tracers are better to visualize the qualitative characteristics of the lymphatic system such as anatomy and morphology of the vessels and lymph node, whereas for quantitative investigations with the goal to measure the speed of the uptake macromolecules are more suitable. Furthermore, it is important that the tracer is stable and that the radioactive marker remains attached to the macromolecule or colloid until the substance is passed into the venous system via the lymphatic duct. With unstable markers there is a risk that the detached radiolabel leaves the interstitium via the venous capillaries, altering the readings on the lymphoscintigram.

Commonly noted sightings in lymphedema include no, or limited, uptake of the injected substance, subdermal spread of the tracer or initial uptake by the lymph vessels but then back flow into the dermis upon reaching the site of obstruction. The axillary and supraclavicular lymph node might be visualized depending upon extent of surgery and anatomical variation. Remarkably, approximately up to 60% of patients treated with an 'complete' axillary lymph node dissection still show a nodal uptake in the axilla on the lymphoscintigram, depending on the tracer used [99].

Different classification schemes to quantify the lymphedema from lymphoscintigraphy have been developed. Lee et al.[100] used an ordinal 4 point scale for six different characteristics (*See table 1*).

Another group has suggested the Transport Index Score (TIS). The TIS is a semi quantitative measurement of transport of the radioactive tracer from the injected place by means of a relative value score (0–9) for 5 different markers. Therefore the TIS ranges from 0 (normal) to 45 (pathologic) and is calculated as:  $TIS = K + D + 0.004T + N + V$ . Where  $K$  = degree of transport delay,  $D$  = degree of dermal backflow,  $T$  = timing of radionuclide appearance in regional lymph nodes (in minutes normalized for 200 minutes, the maximum delay accepted for lymph node appearance),  $N$  = demonstration and intensity of lymph nodes, and  $V$  = demonstration and intensity of lymphatic collectors[101].

Feature	Grade I	Grade II	Grade III	Grade IV
Degree of lymph node uptake	Decreased	None	None	None
Presence of dermal backflow	None	Present in: a) <half of each limb b) >half of each limb	Present	Poor or no visualisation
Visualisation of collateral lymphatics	Good	Decreased	Poor	None visualised
Visualisation of the main lymphatics	Decreased	Poor to no visualisation	None visualised	None visualised
Clearance of tracer from the injection site	Decreased	Greater decrease	No clearance	No clearance
Edema	Mild, easily reversible	Moderate, reversible with effort	Moderate to severe, minimally reversible or irreversible	Severe, irreversible

**Table 1. Lymphoscintigraphy staging as derived from Lee and Bergan (2005)[100]**

Lymphoscintigraphy can be a helpful tool differentiating between lymphedema and other forms of edema, but doesn't always clearly distinguish between primary and secondary lymphedema.

Although lymphoscintigraphy becomes more available in the western world, interpretation still requires training and expertise. Additionally, patients are exposed to small amounts radioactive

material and gamma rays. Furthermore, patients with or at risk of developing lymphedema are prone to infections and therefore advised against having injections into the affected limb.

Lymphoscintigraphy involves such injections and therefore may be considered to be an unnecessary risk. Finally, lymphoscintigraphy is significantly more expensive (up to €700, - per test) than the commonly used assessment methods (circumference/volume and self-assessment). There is no research investigating cost-effectiveness of implementing lymphoscintigraphy in the routine follow-up of all breast cancer survivors or specified risk groups.

### **1.8.6 Bioimpedance spectrometry (BIS)**

Bioelectrical impedance analysis is a widespread non-invasive technology within medicine: a small low-frequency electrical current (conventionally of 200–800 mA) is passed through (a part of) the body through a set of cutaneous electrodes and resistance to that current is measured.

The current is applied through electrodes attached to the skin at the extremes of the area of interest, so the case of BCRL the arm. Two additional electrodes are placed to span the region of interest and detect the impedance to the flow of current. The impedance is the resistance of the tissue at a given frequency and is inversely related to the volume of the tissue water content:

$$V = \rho \frac{L^2}{R}$$

In which V is the volume of the water content,  $\rho$  the specific resistivity of the conductor or in BCRL the arm, L the conductor length and R is the measured resistance. Due to different total water concentration different tissues in the body have different levels of resistance, which results in a characteristic flow of the electric current. Both intracellular and extracellular fluids are conductive, therefore exhibiting a certain resistance. The cellular membrane acts as a capacitor. In theory, the current passes through the extracellular fluids or interstitium at low frequencies and does not penetrate the cell membrane as the flow of the current through the cell is blocked. At higher frequencies the current can penetrate the cell membrane and flows through both the extracellular and intracellular space.

Simple "single-frequency" bioimpedance analysis (SF-BIA) measures the resistance (R) at one frequency (commonly at 50 kHz). With a prediction equation based on the resistance index ( $L^2/R50$ ) total tissue water can be calculated, however this technique can't discriminate between intracellular and extracellular water content and comparison with the unaffected control arm is needed. Therefore, to assess lymphedema more accurately the impedance of the tissue must be measured at different frequencies. Impedance measured at low and high frequencies can be used to calculate extracellular water content and total tissue water content based on the resistance index at specific frequencies. Bioimpedance spectroscopy (BIS) is a more theoretical approach of this technique. It uses extrapolated resistance values at zero and infinite frequency to calculate fluid compartments based on regression equations. By assuming a parallel arrangement of the extracellular water content and intracellular water content, the method provides resistance values that can be used to estimate changes in extracellular or interstitial water content caused by lymphedema since there's a redistribution of fluids between the intracellular and extracellular spaces. Therefore, in contrast to other assessment methods, which measures only the total volume of the limb bioimpedance spectrometry is sensitive to changes in extracellular fluid. When applied to the quantitative analysis of lymphedema the pathological accumulation of extracellular fluid is displayed by a decrease in measured resistance. If we assume the intracellular fluid volume is relatively unaffected by lymphedema[102], a ratio for extra-to intracellular volume can be constructed from the measured impedances.

In a particular study, bilateral upper extremity BIS and limb circumference were recorded in healthy control subjects to establish the normal range of extracellular and total limb volume ratios. Consequently a group of patients were scheduled for breast cancer surgery and BIS and circumferences were measured before surgery and at intervals thereafter for 24 months. Up to approximately 20% developed lymphedema in the time period. In all patients BIS predicted the onset of lymphedema up to 10 months before clinical diagnostic criteria (in this case limb circumference), claiming that BIS allows early identification of lymphatic pathology[102]. Although these results are

not supported by others[88], there is a general belief that BIS possesses greater discriminating capacity than direct volume assessment, especially when it comes to the potential of early detection and in monitoring therapeutic impact[103].

Bioimpedance spectrometry is a quick, cost-effective device to assess lymphedema. However, the technique relies upon the use of specified anatomical landmarks for electrode placement and standardized impedance formulas contained within its programmed software which depends on the equipment that is used. Errors might occur in the estimation of the resistance at a frequency of zero and infinite frequency since this depends on the performance of the electronic measuring apparatus and the data modelling procedures used to estimate these parameters from the actual measured data. Furthermore, some suggest that arm dominance[104], age[105] and obesity[106] may influence the outcome of bioimpedance spectrometry and should be taken into account when using BIS to assess lymphedema. The biggest downside of bioimpedance spectrometry remains the need for a control site on the body, often the other limb. Although many have suggested absolute cut-off points, analysis show that although significant the magnitude of the differences between healthy and affected tissue is too small to implement in clinical practise[104].

Finally, the BIS technique has been investigated to assess lymphedema of the extremities or total body, but only one small scale study has tried to assess tissue water content in the breast self with BIS[107]. The current through each quadrant of the breast is measured separately at the halfway point between the electrodes placed near the areola and the 'outer edge' of the breast. The first problem is that breasts are different in size and shape, especially after breast cancer surgery and clear anatomic landmarks are missing. Therefore the 'outer edge' of the breast and former place of the areola in case of a complete mastectomy might be hard to indentify and very dependable on the observer. The other problem is that  $\rho$  (specific resistivity of the conductor) and L (distance between electrodes) in the formula not only vary significantly between different patients but also for each quadrant of one breast. Far more research needs to be done and better tools need to be developed

before BIS will be an adequate way to assess lymphedema of the breast tissue. Since BIS is predominantly used in a research setting costs of this assessment method vary significantly. Finally, the device should not be used in people with pacemakers or implantable defibrillators.

### **1.8.7 Computed Tomography (CT)**

CT imaging has been shown to be highly sensitive (97%) and specific (100%) in confirming the diagnosis of lymphedema[108], but does not have the detailed imaging ability available with other methods[109]. A significant increase in subcutaneous tissues can be spotted on cross-sectional analysis of lymphedema patients, however density measurements (in Hounsfield units) do not correlate with lymphedema[77]. Therefore CT can only assess lymphedema when the disease has resulted in a significant volume increase of the limb. Furthermore, patients are exposed to a relative high dose of radioactive radiation and CT doesn't provide any advantage over MRI, therefore it is recommended to only use CT when MRI or lymphoscintigraphy is unavailable. The cost of one CT-scan including analysis by a radiologist is approximately €250,- in medical centres with a own CT-scan.

### **1.8.8 Tonometry**

Tonometry measures the resistance of the tissues to compression and therefore provides an indication of tissue softness or fibrotic induration. It does this by measuring the tissue's resistance to compression by an applied weight (often 200 gram) at the tissue above the deep fascia by using a displacement gauge. The higher the resistance to compression, the higher the estimated level of lymphedema is. Nowadays, mechanical tonometers are replaced by digital indurometers, which are more accurate and easier to read. Although proven to be an effective way to assess lymphedema[107], tonometry does have several shortcomings. Firstly, it does not measure absolute amounts of fiber or changes in it. Preferably you would like to have a baseline (pre-treatment) indication of tonometry of the affected limb and track the changes as the patient is treated.

Secondly, it is also important to try to get a contralateral reference point on a normal limb, since absolute numbers yield very little information due to individual differences, hence the results must be compared with contralateral limb, or to measurement changes over time.

Furthermore, when using classical mechanical tonometers the dial must be read while the device is placed on the skin and balanced by the observer[110]. The inconsistency between clinicians or observers is even more influenced by the application of different amounts of pressure to the skin by the clinician[110]. Since the operation of the device is dependant on gravity it can only be used vertically. Finally tonometry relies on the amount of pitting of the tissue, therefore unusable in the early stages of the disease when there are no clinical changes yet, or in the irreversible later stage of the disease when the tissue has become fibrotic and no longer pits. Therefore with respect to the lymphoedematous condition, there are few published data that compare tissue indentation resistance to other relevant lymphoedema related parameters at the beginning of final stage of the disease.

When pitting is present it is important that the reading is at a fixed time after the weight is applied, because the reading changes with time as the tissue pits[110], making the tonometer even less suitable as an objective measurement method. The prices of tonometers range from €50,- for a simple tonometer to €500,- for professional digital indurimeters. After the purchase of the tonometer there are, besides labour, no additional costs.

#### **1.8.9 Dual energy X-ray absorptiometry (DEXA)**

While DEXA is primarily used to estimate bone mineral density in site-specific areas of the body, it is also used to determine lymphoedema in the research setting. Although proven effective DEXA is very rarely used to measure lymphoedema in clinical practice[111-113]. DEXA is a method based on measurement of the weakening of an X-ray beam with two energy peaks by tissue. It estimates the weight of the fat mass, fat-free mass and bone mineral content[111] by differences in densities.

DEXA depends on using the known densities of fat mass, fat-free mass and bone, which makes it suitable to estimate the total volume of the arm as well of the fat-free mass. For each pixel in a DEXA

image, these three mass components are quantified. The fat-free soft tissue mass is the sum of body water, protein, glycerol and soft tissue mineral mass. However, the distribution of the lipid, bone mineral and non-lipid soft tissue within the volume projected onto the image pixel is not known. Theoretically an increase for fat-free mass over the contralateral arm could indicate the presence of lymphedema.

However, the two dimensional DEXA image only provides information about the amount fat free mass of a certain body compartment in grams per unit area, since DEXA does not have the ability to measure tissue thickness. Thus, DEXA systems cannot tell the difference between thick low density soft tissue and thin high density soft tissue, information that can be useful in determining the localisation of the tissue fluid retention.

Since DEXA scans are depending on set reference densities, it occasionally occurs that in some patients some of the soft tissue of the upper arm is marked as bone by the computer software[112]. Secondly, all densities are calculated through a series of equations that accompany the instrument. The instrument uses mathematical algorithms to estimate the body composition components measured by the DEXA using various physical and biological models which are based on assumptions regarding hydration, potassium content, and tissue density. These assumptions can vary between DEXA instrument manufacturers[114]. Furthermore, some suggest that these reference values also slightly differ for age, gender, race, height and BMI[112], although this statement is not supported by others[115].

Other disadvantages are inter-observer variations and, albeit negligible, radiation is used and therefore the relative contraindication of scanning pregnant women. Finally, DEXA is relatively expensive compared to other lymphedema assessment methods and requires training to operate and interpret. DEXA is a special imaging modality that is not typically available with general use X-ray systems because of the need for special beam filtering and near perfect spatial registration of the two attenuations.

Besides the relative low cost (€75,-), the other advantage of DEXA is that it can be performed on patients immediately after surgery, as well as on patients with severe skin disorders, elderly, and very ill patients.

### 1.8.10 Tissue dielectric constant

In tissue dielectric constant measurement (TDC) an electromagnetic wave is sent into the tissue by an open ended coaxial probe, creating an electromagnetic field in the tissue. Depending on the relative permittivity of the tissue, or dielectric constant of the tissue, the alterations in magnitude and phase of the electromagnetic wave that travels through the tissue vary. These alterations are measured by another part of the probe and sent to the computer to be processed. The dielectric properties of a tissue responsible for this wave shift are directly influenced by the total amount of water in a tissue.

The electrical properties of any material, including biological tissue, can be broadly separated into two categories: conducting and insulating. In a conductor, the electric charges move freely in response to the application of an electric field, whereas in an insulator or dielectric, the charges are fixed and not free to move. In practice, most materials, including human soft tissue, display characteristics of both because they contain dipoles as well as charges that can move, but in a restricted manner. Skin and soft tissue can partially be considered a dielectric: an electrical insulator that can be polarized by an applied electric field. The relative permittivity ( $\epsilon_r$ ) of a dielectric is defined as the factor by which the capacitance of the tissue, in other words the ability to store an electrical charge, increases when the volume between and around its plates is filled with the dielectric as compared with free space ( $\epsilon_0 = 8.8541 \times 10^{-12}$  F/m):

$$\epsilon_r = \frac{\epsilon}{\epsilon_0}$$

In which  $\epsilon$  is the measured complex permittivity. The relative permittivity ( $\epsilon_r$ ) of a vacuum (at room temperature and 1 kHz) is by definition 1. The relative permittivity of a tissue depends on the applied frequency and the temperature[116]. In contrast to bioimpedance spectrometry that measures the

conductivity of the tissue, tissue dielectric constant measurement merely registers the alternations of the electromagnetic wave by the polarisation of the tissue by the probe. The measured quantity is the net result of the interaction of the electromagnetic wave with the water molecules and thus dielectric constant of the multiple layers of tissue.

In research, lymphedema assessment is predominantly done with a commercial open-ended coaxial probe that creates a high frequency of 300MHz. At this frequency the interaction between the wave and tissue is mainly dominated by dipolar relaxation of bulk water molecules[117, 118], either free or bound on the surface of macromolecules. The TDC at this frequency is directly proportional to the intracellular and extracellular tissue water content[119]. At high frequencies the measurement depth is mainly determined by the probe size. Depending on probe size the electromagnetic wave can reach into the subcutaneous fat. The effective depth of the probe is defined as the tissue depth at which the electric field has been attenuated to  $1/e$  (approximately 37%) of the surface value[119]. Even though the upper layer of the skin, the superficial stratum corneum, is very thin it dominates the tissue dielectric constant at low frequencies. However, at the frequency of 300MHz the measurement results are largely influenced by the water in deeper structures. Studies show that, for frequencies under 10 kHz, the share of stratum corneum in the TDC reading of skin is around 50%, but at 100 kHz drops to around 10%[120]. Since the water content of the stratum corneum becomes irrelevant at a frequency of 300MHz the complex permittivity or in other words TDC will be predominantly influenced by the tissue water in deeper lying layers such as the dermis and top of the subcutis. This makes the TDC method an adequate tool to assess lymphedema[121].

Tissue dielectric constant measurement has proven to be a quick, cost-effective device to assess lymphedema. In contrast to many other methods including bioimpedance spectrometry the outcome does not strictly depend on specific anatomical landmarks, reducing the inter-observer bias and making the results reproducible. Secondly, the machine is straightforward and doesn't need any training to operate, thus after the purchase (of €750,-) there are little additional costs.

Since the same probe emits and receives the electromagnetic wave, the TDC measurement is a localized technique without the need for a fixed trajectory as in bioimpedance spectrometry. This unique feature makes the TDC measurement method theoretically extremely suitable to detect changes in tissue water content of the breast tissue.

Currently there is not sufficient evidence to support the hypothesis that TDC measurements can do without control measurements on the contralateral side. The outcome of the TDC measurement depends the age and BMI of the patient[122, 123], therefore more research with a greater population needs to be done to determine a clinical TDC cut-off point.

## 1.9 Therapy

Lymphedema is a chronic condition which requires lifelong treatment. The disease has many treatment options that have demonstrable efficacy for the reduction of edema volume and the prevention of fluid accumulation. Early recognition of lymphedema related symptoms are essential in the treatment of lymphedema. Preferably the condition is diagnosed while still in a reversible stage, before fibrotic tissue starts to form. Therefore, an assessment method that can detect early changes in tissue water content, associated with lymphedema, would be of great value. Non-operative therapy options are mainly focussed on reducing the swelling, recovery of the function of the extremity and improving the quality of life of the patient. They can take away the symptoms for a period of time, but very rarely cure the lymphedema. On the other hand, if treatment is discontinued, new exacerbations of lymphedema will present causing impaired limb function, skin defects and possibly infectious and malignant complications.

Aside from physical discomforts, lymphedema can also seriously influence the psychosocial function of the patient. Psychological dysfunction can even worsen the lymphedema due to feelings of hopelessness, disgust and social isolation[124]. Therefore, education and counselling during the treatment of lymphedema must not be underestimated.

Furthermore, continuous lymphedema treatment can be expensive, time consuming and often involves a multidisciplinary approach. In general, there is a lack of high quality randomized controlled trials evaluating the effect of different treatment modalities in lymphedema management. It is therefore recommended that each patient's response to treatment is closely monitored so that early adjustments can be made. Interdisciplinary communication and also clear delivery of information about the treatment to the patient is considered to be essential. This increases the commitment of the patient to the program when results are minimal or absent. For that reason, patient support groups may be helpful in improving patient compliance.

### **1.9.1 Compression therapy**

In the centre of most lymphedema therapy stands external compression. Compression therapy aims to decrease the interstitial fluid production and tries to transport the excessive lymph fluid in the extremity back to the main circulation. The spectrum of external compression consists of elastic sleeves, stretch compression bandages and pneumatic compression devices.

Compression therapy with elastic sleeves is very rarely used as an isolated treatment, but shows great benefits when it's employed after decongestive treatments[125, 126]. Compression sleeves do oppose capillary filtration and prevents backflow to the superficial lymph system, diminishing clinical relevant lymphedema. Additionally, elastic sleeves contribute to the venous return flow, helping patients with a venous stasis component to their disease.

Elastic sleeves can be divided into 'daytime' and 'nighttime' sleeves. Both can be ordered in standard sizes, but frequently they are custom made based upon meticulous measurements taken by a professional. Depending on the severity of the lymphedema, sleeves divided into four classes with a pressure of 20 mmHg to 60 mmHg can be fitted. Normally the tightness of 'daytime' sleeves decreases gradually as it comes closer to the axilla. The more expensive 'nighttime' sleeves are foam padded and equipped with straps to adjust the amount of compression. There are no publications that have investigated treatment effectiveness between the two groups. The major downside is that normal compression sleeves need to be replaced every 4 – 6 months since they lose a significant amount of their elasticity. Furthermore, it has been shown that effective compliance with compression sleeves is variable because they can be uncomfortable, difficult to put on and cosmetically undesirable. Since the applied pressure by the compression sleeve depends on the severity of the lymphedema, patient satisfaction and compliance can significantly benefit from early detection and induction of the therapy. This underlines the need for an objective measurement method that can detect the disease in an early stage.

Beside these discomforts, serious complications can occur during compression treatment including dermatitis as well as the induction or aggravations of the swelling distal to the sleeve. Wearing compression sleeves during skin infections or wounds is contraindicated.

An alternative to compression sleeves is compression bandaging. Based upon the same principles, multiple layer short stretch bandaging (in combination with a compression sleeve) almost doubles the reduction in volume over a greater period of time than just a compression sleeve solitarily[127]. The only major drawback for multilayer bandaging is that the time consuming procedure ideally has to be repeated daily for multiple weeks[128].

Another treatment method is intermittent pneumatic compression (IPC) where a sleeve envelops the limb and inflates and deflates at different cycles and pressures. Intermittent pneumatic pumps are available in single- and multiple-chamber sleeves and even sleeves plus upper body suit. The rationale behind the body suit is lymph clearance from the larger, more proximal vessels providing space for the lymph fluid in the affected area to drain to[129, 130]. Intermittent pneumatic compression systems with multi-chamber pumps mimic the motion made manual decongestive treatments by inflating the sleeve from distal to proximal, thereby producing a wave of pressure that ascends the extremity. It has been suggested that this allows the retained fluid to be brought to the more proximal and deeper functional lymphatics supporting the return flow to the venous circulation. Claims have been made that intermittent pneumatic compression pumps effectively remove excess fluid from the extremity and can be used as a primary treatment[129], however, it has been disputed[131]. Significantly increased reduction of lymphedema (measured by water displacement) is also reported when additional IPC is used in the treatment program[132, 133], however others don't support this view [134]. Therefore the real contribution of single IPC, or, in addition to conservative decongestive lymphatic therapies, remains a topic of debate. Although some are convinced of the positive effect of IPC, there are not any established guidelines regarding optimal pressure ranges, inflation/deflation cycles, length or frequency of individual pumping sessions. A potential method to

settle this argument is suggested here for an objective method to quantify minimal changes in fluid retention.

Intermittent pneumatic compression is relatively safe and free of complications, nevertheless initiation or aggravation of truncal and even genital lymphedema should be closely monitored. At high pressure, peripheral lymphatic damage is a potential complication. As with the compression sleeves there is a risk of skin defects and aggravation of the lymphedema at the distal end of the arm envelop. Additionally, more quickly than with compression sleeves, fibrotic tissue can form in this particular region thereby further compromising the lymphatic outflow. Hypothetically, use of these pumps is advised against in patients with local or proximal malignancies because of concern of possible metastasis. Another concern is worsening of congestive heart failure or induction of congestive heart failure in a patient with severe coronary artery disease[135], although these statements are not supported by any evidence. Obviously, in cases of limb ischemia, compressive therapy, which can compromise arterial blood flow and promote severe ischemia and necrosis, is contraindicated.

Compression therapy is considered by most as an essential part of lymphedema treatment. Despite this there is no conclusive evidence to support this claim. When looking for evidence-based confirmation of outcomes and a composite protocol of compression therapy, there is disappointingly little corresponding evidence. There are considerable inconsistencies in defining and measuring lymphedema, small sample sizes, and a lack of blinding of participants and assessors, all of which leads to inadequate power and a high likelihood of errors. Therefore it's not surprising that conclusions are contradictory and ambiguous.

### **1.9.2 Manual Lymph Drainage therapy (MLD)**

(Complex) physical therapy is often added to the compression therapy regime and is designed to improve lymphatic drainage. The therapeutic intervention in the first phase includes manual lymphatic drainage, sometimes in combination with exercise together with meticulous skin care prior

to compressive garments. The second phase often consists of maintenance treatment at home.

Modalities of maintenance therapy are often not well defined, but can consist of self-massaging, massage treatment from relatives and wearing extra compression sleeves.

Manual lymph drainage is a unique massage technique that claims to cause lymph tracts to contract more often and drainage of the lymphatic fluid away from congested regions, and subsequently into the deeper lymphatic system.

Manual lymph drainage is a collective title for many different techniques, but is most commonly used to describe (a derivative of) the Dr. Vodder technique[136]. With the Vodder-technique the skin stretched and torqued in a specific manner with constant change in pressure, hypothetically moving the interstitial fluid in the skin and reducing fibrotic induration. This ambulatory treatment is normally performed on a daily basis for 1 to 6 weeks starting with massaging the contralateral side of the trunk then working from proximal to the distal end of the affected extremity.

The effective result of manual lymph drainage in the current literature is highly dependable on the lymphedema assessment method, included patients, treatment duration and schedule and definitions of successful treatment. Although many agree that MLD is an effective treatment method in patients with established lymphedema of an extremity, success rates vary significantly. In a large study including 149 patients with upper-extremity lymphedema (98% secondary lymphedema) a significant reduction of almost 70% was measured by circumferential volume assessment directly after the MLD treatment period (plus compression therapy) in 131 patients[137]. After six months and twelve months, 82% patients who initially benefitted from MLD treatment still showed a decrease of approximately 55% in volume of the affected arm. The reduction of lymphedema directly after MLD treatment is supported by others[138], albeit with lower success rates. Long term follow-up figures in a study conducted in 535 patients by Vignes et al.[138] are less optimistic, especially after patients abandon MLD treatment. Volume reduction of MLD treatment alone has not been properly investigated: no study reported on MLD as a single modality versus no intervention. The maximum result is achieved if the compression garments are not removed until the subsequent MLD

session[139]. No randomized controlled trials have been conducted to investigate the effect of manual lymph drainage as a prophylactic treatment.

MLD is considered to be a safe treatment modality, however, caution is needed when treating patients with relative contraindications including malignant tumors, thrombosis or venous occlusions, hemorrhage, acute enuresis and major cardiac pathology. The reported adverse events are: cerebrovascular accidents, embolisms, haematomas, nerve damages, skin ulcers, pseudo-aneurysm, thyrotoxicosis and various pain syndromes, although because of small numbers the cause-effect relationship remains unclear[140].

In conclusion, it's believed that continuous MLD in combination with compression therapy with, or without, maintenance self massaging in the second phase decreases the chance of developing lymphedema in at risk patients[137-139], but some small scale research suggest that single MLD alone does not help[92, 141]. The effect of MLD may even be negligible compared to other treatment modalities implemented in the therapy of the patients[142, 143]. Furthermore, no study has evaluated patients' treatment preferences or the effects of the treatment on quality of life. The lack of consensus on the efficacy MLD is a result of different treatment schedules, control groups and follow-up, but most important the way different researches assess and define lymphedema.

### **1.9.3 Physiotherapy, exercise and elevation**

Physiotherapy often includes, or is confused with, manual lymph drainage in most papers. Besides MLD most physiotherapists believe that (assisted) dynamic exercises can decrease the volume of a lymphoedema affected limb. The hypothesis is that the activation of the muscles surrounding the lymph vessels will stimulate the flow along the valved lymphatic system and even improve protein resorption. Secondly, dynamic training claims to prevent soft tissue contractures, which can obstruct lymph flow. One of the other mechanisms that is believed to have a positive effect on decreasing lymphedema is the drop in intrathoracic pressure due to deep inspiration followed by expiration, suggesting that the cardiopulmonary load of the exercise also contributes to improved lymph

clearance. A major concern is that the exercise will induce an increased blood flow to the treated limb, possibly exacerbating the lymphedema.

Despite these theories the reported results are disappointing. Most studies underline the beneficial contribution of exercise but only small decreases in limb volume are reported. In a study investigating 10 minute arm exercise in combination with a deep breathing regime a decrease of 5.8% and 9% in total limb volume was reported after respectively 1 week and 1 month after start of the exercise treatment. Patients claimed to experience significant improvements in perceived limb size and heaviness as well as improved functional movement[144]. In another study using resistive exercises with hand weights stimulating the latissimus dorsi, biceps and triceps the decrease in arm volume after 24h was 1.0% for patients treated with additional compression therapy and 0.7% in the group without[145]. Subjective self-reports in this study did not show any difference. A second study using more or less the same treatment modality in a small group of breast cancer related lymphedema patients reported a limb volume reduction of 2% and significant better physical function and vitality[146].

Despite the minimal volume reduction, most patients report improvements in range of movement of the arm, perceived limb volume, heaviness and tightness. It remains unclear whether this subjective improvement can be attributed to the reduction in volume or increased physical fitness.

Furthermore, it's not unlikely that the long term measured reduction in limb volume is a result of a decrease in fat mass caused by the exercise. This hypothesis is supported by a randomized controlled trial conducted in 21 patients with breast cancer related lymphedema[147]. The intervention group, patients on a diet with an energy deficit of 1000 kcal per day from habitual intake, showed a significant decrease in total body weight and affected arm volume after 12 weeks. Significant weight loss, especially in individuals with a high body mass index, is believed to be a considerable factor in the management of lymphedema. However, the percentage of limb volume reduction as a result of the decrease in extracellular fluid rather than fat tissue remains unclear. This highlights the need for a tool that can objectively assess the tissue fluid levels.

Although elevation is not considered a main treatment option in the management of lymphedema, it can be used as adjunctive therapy. The thought behind the mechanism of elevation is that elevating the extremity decreases intravascular hydrostatic pressure, therefore increasing the resorption at the venous end of the capillary and thus reduces the amount of lymph load. Despite recommendations by some physicians there is no hard evidence proving the efficacy of elevation in the treatment of lymphedema. The major disadvantage of limb elevation is that continuous elevation during the course of the day can impede the function of the individual perhaps even more severely than the lymphedema itself and it will discourage exercise. Elevation of the arm at night during sleep is bound to fail and practically impossible.

#### **1.9.4 Pharmaceutical therapy**

In normal treatment of breast cancer related lymphedema there is no place reserved for pharmaceutical treatment. Overall the results of drug therapy in the management of lymphedema have been very disappointing. Various pharmacologic agents, most of them with the goal to break down the protein accumulation in the interstitium, have been investigated in addition to compression and drainage therapies.

Some claim that benzopyrones like coumarin derivatives (5,6-benzo-[a]-pyrone) have a favourable effect on lymphedema by limiting the fluid filtration rate rather than stimulating drainage.

Benzopyrones are thought to control proteolysis by increasing the protease activity of cutaneous macrophages, which increases protein degradation, thus resulting in a reduction of extracellular tissue water content. Coumarines are also believed to reduce the vascular permeability and therefore lowering the capillary filtration resulting in a diminished outflow of proteins and fluid.

Finally, it's claimed that coumarines have a positive effect on the immune system by increasing the T-helper/T-suppressor ratio, stimulating NK cells to suppress the production of superoxide and hydrogen peroxide in by monocytes, thereby enhancing protein reabsorption[148]. There is insufficient data to draw any conclusions on volume reduction and secondary outcomes such as

improvement of quality of life and reduction of pain caused by benzopyrones. Although some randomized placebo-controlled crossover studies do report a slow but significant reduction of various types of lymphedema[149-151], but others do not endorse their beneficial effect[152, 153]. Furthermore, for all these studies long term follow up is lacking and reports that benzopyrones can have a hepatotoxic effect cause valid concern[153, 154].

There are also claims that other flavonoids, such as Diosmin, Hesperidin, Ruscus Aculeatus or the combination drug Cyclo-Fort, normally used in chronic venous insufficiency, have a small therapeutic benefit in the treatment of (severe) lymphedema[155-158]. These naturally occurring drugs are believed to have protective effects on vascular endothelium and improve the microcirculation by decreasing the number of macromolecules leaking from the blood vessels. Despite promising results there are no large randomized controlled trials with sub group analysis to confirm this belief.

Therefore, these drugs don't have a role in the common treatment of lymphedema. Unfortunately, there is also no data about the long term effects or potential toxicities of these agents. It is also important to note that flavonoids can cause gastrointestinal discomfort in up to 22.6 % of all patients[149].

Diuretics are contraindicated as they only result in marginal improvement and can potentially lead to increased fibrosis due to more protein accumulation. The use of antibiotics is indicated in the treatment of infections, such as cellulitis and lymphangitis, but no data exists that support the use of antibiotics as standard treatment in lymphedema.

Alongside drugs, nutritional supplements have also been evaluated for the treatment of lymphedema. Zinc[159], selenium[160], triglycerides[161] and Vitamin E in combination with pentoxifylline[162] have been associated with reduction of lymphedema, although never investigated in large well conducted trials. Hard evidence for these claims is missing because of small number, absence of control group, different treatment modalities and lack of objective measurement method and consensus on the definition of lymphedema.

Interestingly enough, many studies reporting on the effect of drugs and nutritional supplements demonstrate a significant reduction in reported discomfort, heaviness and perceived size in all patients including most placebo groups. This underlines the need for a tool that can assess lymphedema in objective and quantitative manner.

### **1.9.5 Surgical treatment**

Generally surgical treatment of lymphedema is reserved for patients who are unresponsive to conservative treatments. The surgical procedures can be categorized in two major groups: debulking of the affected tissue and reconstructive surgery with the aim to restore the lymphatic function. The purpose of debulking procedures is to reduce size and weight and improve mobility and function of the affected limb by removing the excess of lymphedematous skin and subcutaneous tissue. Debulking procedures are not designed to directly address the dysfunction of the lymphatic system and the underlying pathology remains, therefore there's a high chance that the lymphedema may return. Initially, debulking surgery consisted of an aggressive approach in which all of the soft tissue including the overlying skin on top of the deep fascia was resected and the newly created surface was covered by skin grafts. Subsequently the procedure was modified and musculo-cutaneous flaps were used to cover the defect[163]. Although considered beneficial in the treatment of severe lymphedema[164, 165], the true efficacy of surgical debulking remains unknown since most research is hard to compare due different patient selection, surgical approach, additional postoperative treatment and primary outcome. The wide array of used surgical approaches reflects the fact that none of these procedures provides a truly satisfactory solution. Furthermore, information about the long term follow up is missing. Therefore the invasive surgical debulking procedures don't have a place in the standard treatment of lymphedema and should be reserved for patients with unacceptable subcutaneous adipose hypertrophy and fibrosis that is seriously compromising the quality of life. Especially since surgical debulking can be accompanied by serious complications including the potential obliteration of the remainder of the cutaneous lymphatic system. Other

adverse events and complications may be sensory loss of the skin, necrosis of the skin or graft, skin ulceration and keratosis, infections, deep vein thrombosis, hypertrophic scars and contractures and subsequently aggravation of the lymphedema distal to the resected area[166]. Finally, depending upon the extent of the procedure, the patient can be left with a significantly deformed limb with the risk of psychological problems and a diminished quality of life.

Less invasive surgical liposuction of lymphedematous tissues has been reported to be more effective than conventional surgical debulking procedures with a reduced risk of complications. The published percentages of limb volume reduction after 12 months differ from 18% to 118%[167-169]. This wide range of success depends on surgical technique, patient selection and additional postoperative compression treatment modality. Among the complications of liposuction of lymphedema erysipelas is the most common[169].

The main goal of microsurgical reconstruction of the lymphatic system is to return the lymphatic flow to normal or bypass the obstruction in the lymphatic system. This can be achieved by creating lymphatic vessel-venous ('lymphaticovenous') shunts or autologous vessel transplantation in various configurations between vessels of 0.3 – 0.8 mm. These shunts are constructed by a direct lymphaticovenous anastomosis with[170-172], or without, an intravascular stent[173-176].

Lymphatic bypasses can be made by connecting lymphatic collectors to the distal and proximal side of the obstruction with a venous grafts[177], a single[178] or multiple[179] autologous lymphatic vessels. Only patients with functional lymphatics distal of the obstruction are eligible for these microsurgical procedures. Additionally, lymphatic bypasses are preferred over lympho-venous shunts in patients with venous hypertension[177]. In a retrospective 5-year follow up of 665 patients Campisi considered the lymphatic bypass procedure successful in 83% of all patients with a permanent mean volume reduction of 67%[177]. Numbers that are supported by another study[178]. Studies investigating the effect of lympho-venous shunts describe a mean reduction of limb volume of 2% – 60% after 12 months with an initial absolute response rate varying between 73 and 95%[171, 173, 174, 176]. Long-term follow up shows slightly better results when stents were implanted[170,

171] or when the reconstruction procedure was combined with debulking surgery[176]. It's important to note that these studies are highly incomparable because of various microsurgical lymphatic reconstruction techniques, patient selection, severity of lymphedema at the baseline and time from initial diagnosis of lymphedema to the lymphatic reconstruction surgery. Furthermore, many different lymphedema assessment methods were used to determine the success or the procedure. In a group of 23 mastectomy and 9 BCT patients diagnosed with BCRL and treated with a lymphatic bypass a reduction of limb volume calculated from circumference measurements was reported in 73 % of all cases, however only 42% of all patient noticed an improvement of quality of life[176]. This raises the question about the definition of success in these procedures. Additionally, complementary therapeutic options were heterogeneous, not comparable, and lacked a validated method of effect-assessment.

Regardless of the operation technique used, there is a significantly lower rate of cellulitis in patients treated with lymphatic reconstruction[176, 177]. Furthermore, although lympho-venous shunts can help reduce the severity of lymphedema in most patients, it does not cure lymphedema. It's recommended to immediately continue with ongoing conservative compression treatment, which makes definitive conclusions regarding the direct benefit of the surgical procedure difficult.

Finally, to know the exact location of the obstruction in the lymphatic system lymphoscintigraphic imaging is required, but improvements seen on the post-operative lymphoscintigram don't carry any prognostic values about eventual volume reduction.

There is no hard evidence that the construction of prophylactic lymph shunts during cancer treatment can diminish the incidence of lymphedema.

### **1.9.6 Heat therapy**

Heat increases the blood flow by stimulating the thermoreceptors in the skin and deeper tissues. Besides reducing muscle tension by reducing the viscosity of collagen, heat also results in vasodilation increasing circulation and cellular metabolism. In theory, this increased circulation

results in a higher supply of cutaneous macrophages stimulating the breakdown of (extracellular) proteins and a higher potential to reuptake proteins and debris at the venous side of the capillary. Heat therapy does not increase the lymphatic flow but is believed to accelerate the venous refill time[180], however others suggested that heat increases the protein uptake by the lymphatic system. An in vitro model showed a significant increase in lymphatic transendothelial transport rate with increasing temperature[181].

Although heat therapy has proven to be successful in the regression of inflammation in other diseases, the effect of heat therapy in the treatment of lymphedema is missing solid evidence. Most research is dated, lacking consensus on treatment modalities and assessment method[182]. In a more recent small scale study no benefit was noted of additional heat therapy when comparing mechanical lymph drainage alone and lymph drainage combined with heat therapy[183].

### **1.9.7 Low level laser therapy**

Low-level laser therapy claims to alter cell and tissue formation via non-thermal photochemical reactions in the cell. The low-level laser source generates a single wavelength light and is used in a wide variety of clinical conditions from acne to myocardial infarctions. In vitro studies suggest that low-level laser therapy stimulates collagen production and reduces prostaglandin synthesis lowering the risk of tissue inflammation. Low level laser treatment is also believed to increase lymphatic flow by encouraging lymphangiogenesis and stimulation of macrophage cell activity[184]

In a study of 61 post-mastectomy patients 2 cycles of low-level laser therapy was found to be effective in reducing the volume of the affected arm and extracellular fluid in almost one third of all patients 3 months after treatment[184]. These positive results are supported by other smaller scale randomized controlled trials albeit it with different treatment modalities, laser intensity and definition of success[185-187]. One study investigating the long term effect of low level laser therapy showed permanent reduction of 29% of arm volume after 36 months but the subjective symptoms returned to the pre-treatment levels[188]. There is strong evidence that suggest low level laser

therapy can contribute in the management of BCRL. Nevertheless, larger randomized controlled studies with wider subgroup analyses and longer follow-up are necessary to assess the real value of low-level laser therapy.

#### **1.9.8. Skin care**

Obviously good hygiene can prevent infections. Meticulous skin care can significantly diminish the risk of cellulitis, lymphangitis and erysipelas. Although regular cleansing and drying of the lymphedematous areas is essential, it entails the risk of dehydration of the skin resulting in lesions. Verrucous skin surface is associated with an increased risk of infection due to the potential bacterial build up in the quarries. Therefore moisturizing ointments may be a valuable asset in daily skin care. Signs of infections should be treated with antibiotic treatment as soon as possible.

## **2.0 AIMS OF THIS STUDY**

## 2.1 Aims of this study

In the early, often reversible, stage of lymphedema patients can experience subjective changes in the affected area. However, with the traditional tools available in daily practice the lymphedema often remains clinically undetectable. Currently available lymphedema assessment methods all have their shortcomings. Circumference and volume assessment using girth measurements are not accurate and lack a common consensus resulting in a wide range of definitions for lymphedema. Furthermore, it is impossible to detect small changes in tissue water content with this method. More accurate volume assessment methods such as optoelectronic perometry and the water displacement technique are respectively expensive and cumbersome and therefore hard to implement in daily practice. Ultrasound, MRI, CT and lymphoscintigraphy are more sensitive in the detection of early onset of BCRL but expensive and require training to operate. Self assessment is not objective and highly dependable on the questionnaire used.

This underlines the need for a sensitive objective measurement method that can assess lymphedema in a subclinical stage. A sensitive objective method will not only help identify risk factors associated with lymphedema, but also improve treatment. Especially since women diagnosed with lymphedema in an early stage of the disease benefit more from the currently available lymphedema treatments and have better prospects[45].

In this study, we aim to demonstrate that measuring tissue dielectric constant using the MoistureMeter-D is an effective method to detect tissue water changes and has the potential to provide an inexpensive tool to measure the early onset of breast cancer related lymphedema of the arm and breast in daily clinical practice.

Second to this, we will investigate the correlation between the novel TDC method and the frequently used arm volume measurements and self-assessment questionnaires.

If tissue dielectric constant method has been proven to be an adequate way to quantify lymphedema it can be used in a variety of prospective trials monitoring the treatment of lymphedema. This method does have the potential to closely monitor the (long term) outcome of early versus late

intervention with compression therapy and the value of additional MLD or physiotherapy.

Additionally, we aim to provide a clinical TDC cut-off point, compensated for age and BMI, which can be used in the general population of patients diagnosed with breast cancer. We believe this will allow us to diagnose lymphedema in an early subclinical stage after surgery or even identify high risk patients before breast cancer treatment. Eventually we would like to conduct a randomized clinical trial in the future in a group of patients with early elevated TDC levels. In this study we will compare the outcome of intervention in the subclinical stage or even preoperative phase with the currently available lymphedema treatment modalities and late intervention when symptoms of lymphedema start to show.

Secondly, using the TDC we could more accurately identify subgroups of high risk patients (i.e. extend of axillary surgery, radiotherapy and high BMI). In our opinion the TDC assessment method provides a tool that can investigate the numerical correlation between extend of the surgery and the amount of lymphedema.

## **3.0 METHODS**

### **3.1 Patients**

Twenty patients with previous unilateral breast cancer-related surgery were recruited into the study from the local Lymphedema Clinic (Oxford Radcliffe Hospitals NHS Trust, UK). After giving written informed consent, a brief medical history was taken and information about the patients' surgical procedure (date, type of surgery, adjuvant therapies) and details about their attendance at the lymphedema clinic (months between surgery and initial visit, months between initial visit and day of testing, total number of clinic visits and type of treatment they received) were collected. Also information about the treatment they received during these visits (fitting of compression sleeves, manual lymphatic drainage and bandaging treatments) was collected from the patients' notes. (See *table 2.*)

Patients were seen at the start of their clinic appointment before any treatment was carried out.

An inclusion criterion for the study was previous unilateral breast cancer-related surgery. Exclusion criteria were previous reconstructive surgery, and chronic pain or neurological conditions that were unrelated to previous breast cancer surgery. Ethical approval was obtained from the Local Research Ethics Committee and the study was performed in accordance with the Declaration of Helsinki.

### **3.2 Volume measurements**

Eighteen of the twenty patients recruited were assessed using the traditional volumetric method used by the clinic followed by the novel tissue dielectric constant method. All measurements were taken with the patients sitting upright in a comfortable chair. TDC measurements were taken from the dorsal aspect of the arm at the same locations used by the clinic for the circumferential measurements that enable the calculation of the arm volume. Consequently, both circumferential and TDC measurements were performed bilaterally at ten sites along the arm at 4cm intervals, with site 1 positioned at 19cm from the base of the nail of the third finger. Following the circumferential measurements, a single TDC measurement was performed at the same locations on the arm. Arm limb volume was calculated from the circumferential measurements as the sum of the nine segment

Patient	Age	BMI	Surgery*	Side	Tumor grade	Lymph nodes	Lesion size	Mass ** removed	HTx	ChTx	RTx	First visit lymphedema clinic	Follow - up	Nº of visits	Lymphedema Treatment
1.	55	35.8	ULQ	Left	n.a.	15	n.a.	n.a.	n.a.	n.a.	Yes	4	4	n.a.	n.a.
2.	60	31.2	UMQ	Left	1	8	19	126	Yes	No	Yes	30	24	4	Sleeve
3.	70	31.6	Mastectomy	Left	2	0	22	575	Yes	No	Yes	10	102	23	SLD + Sleeve
4.	55	29.4	Mastectomy	Left	n.a.	8	40	1015	Yes	Yes	Yes	36	4	1	Sleeve
5.	66	23.6	Mastectomy	Left	3	3	21	114	No	Yes	Yes	33	84	9	MLD + Sleeve
6.	82	20.9	Mastectomy	Right	1	n.a.	n.a.	52	Yes	No	Yes	24	13	1	Sleeve
7.	49	35.5	ULQ	Left	2	16	50	74	Yes	Yes	Yes	12	2	5	MLD
8.	64	34.3	ULQ	Right	2	5	30	199	Yes	Yes	Yes	12	17	15	MLD
9.	58	n.a.	Mastectomy	Left	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	31	11	2	Sleeve
10.	44	28.8	Mastectomy	Left	3	13	37	1240	No	Yes	Yes	7	17	4	Sleeve
11.	63	24.5	UMQ	Left	1	0	8	43	Yes	No	Yes	13	4	3	SLD
12.	46	25.0	Mastectomy	Right	2	13	100	550	Yes	Yes	Yes	16	12	2	Sleeve
13.	68	18.4	ULQ	Left	2	7	9	65	Yes	No	Yes	27	68	38	MLD + SLD
14.	49	32.4	LLQ	Right	3	8	10	n.a.	Yes	Yes	Yes	4	22	13	MLD
15.	62	39.3	Mastectomy	Right	3	n.a.	32	1110	Yes	Yes	Yes	33	6	2	Sleeve
16.	64	39.2	ULQ	Right	3	23	36	508	Yes	No	Yes	4	43	9	Sleeve
17.	62	24.8	LLQ	Left	n.a.	6	n.a.	n.a.	Yes	n.a.	n.a.	9	20	19	MLD + SLD
18.	68	33.1	Mastectomy	Right	2	n.a.	24	452	Yes	Yes	Yes	6	27	12	MLD + SLD
19.	72	21.8	Mastectomy	Left	3	9	56	n.a.	Yes	Yes	Yes	72	144	40	Sleeve
20.	69	29.0	UMQ	Right	3	n.a.	11	44	Yes	No	yes	37	7	9	MLD + SLD + Sleeve

**Table 2. Patients characteristics.** Age initial surgery, Follow-up is the time in months from first visit to the lymphedema clinic till the day of assessment. MLD: Manual lymph drainage, SLD: self lymph drainage. \*Extent or location of surgery with ULQ: upper lateral quadrant, UMQ: upper medial quadrant, LLQ: lower lateral quadrant, LMQ: lower medial quadrant. \*\*Total mass remove during surgery including mass of axillary lymph nodes in years, BMI in kg/m<sup>2</sup>, tumor grade according to Bloom-Richardson classification, HTx: hormone therapy, ChTx: chemotherapy, RTx: radiotherapy, First visit to the lymphedema clinic is the time in months since.

volumes using the formula for a truncated cone:  $V = h \times \frac{(c1^2 + c1 \times c2 + c2^2)}{12\pi}$ . This assessment of the arm BCRL was followed by TDC measurements in the breast in all 20 patients.

### 3.3 Assessment of Tissue dielectric constant (TDC) measurements

TDC measurements were taken using a portable device called the MoistureMeterD (MMD) that detects the reflected proportion of a 300MHz electromagnetic pulse (*See fig.2*). From this measurement, the dielectric properties of the underlying tissue, and the relative tissue water content (TWC), can be inferred with the resulting TDC values scaled from 1-78 where a value of 1 represents air and 78 represents water (MoistureMeterD, Delfin Technologies Ltd, Finland).

The effective depth of the measurement depends on the size of the probe used, with larger spacing between the two coaxial conductors corresponding to deeper penetration in the tissue, from 0.5mm to 5.0mm. The effective depth of the measurement depends on the size of the probe used, with larger spacing between the two coaxial conductors corresponding to deeper penetration in the tissue, from 0.5mm to 5.0mm. In this study, four different probes were used with respectively effective penetration depths of 0.5, 1.5, 2.5 and 5.0mm.

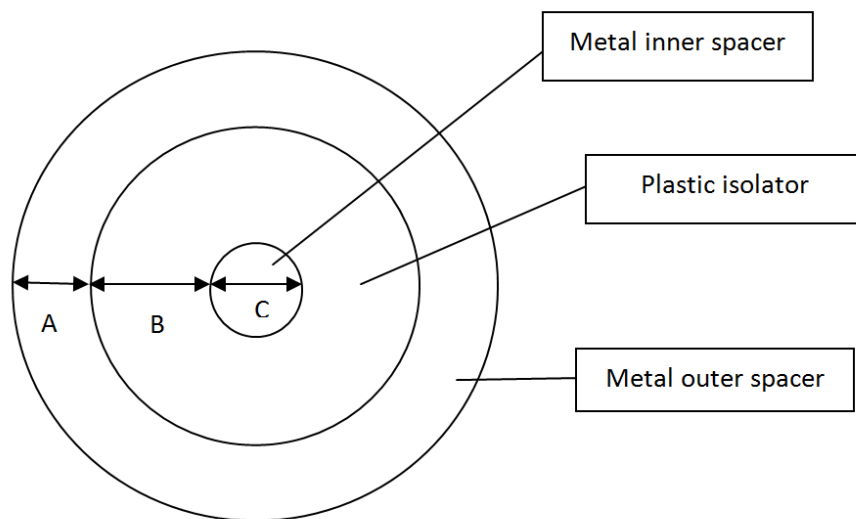
In the arm, we used single measurements with the 2.5mm probe, which has an outside diameter of 23 mm and inner-to-outer conductor spacing of 5 mm, as this has been proven to be effective for measuring TWC in the forearm of lymphoedema patients[123, 189]. The measurements were taken at the dorsal aspect of the arm at the exact same 4cm intervals of the circumference measurement starting with site 1 at 19cm from the base of the nail of the third finger. In the breast, triple measurements with each probe were taken from each of the four quadrants at a diagonal distance of 4cm from the centre of the breast (or nipple where conserved) of the affected breast or chest wall and control breast.



**Fig. 2. The MoistureMeterD (MMD)** developed by Delfin Technologies Ltd, Finland. On the right: the four different probe sizes (from left to right respectively 0.5mm, 1.5mm, 2.5mm and 5.0mm).

### 3.4 Tissue dielectric constant model of the skin

In this research we used the MoistureMeterD to assess the tissue dielectric constant. The MMD consist of a portable main unit, which generates the radiofrequency and analysis the signals of the probe, and four different sized coaxial open-ended probes. The surface area of the probe that touches the skin contains three different areas: an inner “spacer” responsible for transmitting the radiofrequency wave, an outer “spacer” that detects the proportion of the wave that is reflected and a plastic “isolater” separating the two as shown in fig.3. Values calculated by the MMD can be transmitted wireless to software installed on a personal computer (*see Fig. 2*).



Probe size (depth)	A	B	C
0.5mm	1.5mm	1.0mm	5.0mm
1.5mm	5.0mm	3.0mm	2.5mm
2.5mm	4.0mm	5.0mm	5.0mm
5.0mm	7.0mm	17.0mm	6.0mm

**Fig. 3 Top view of probe head**, where C is the transmitting inner metal spacer and A is the outer spacer detecting the electromagnetic field. The isolating space between the transmitting and receiving end determines the measurement depth.

The electrical properties of any material, including biological tissue, can be broadly separated into two categories: conducting and insulating. In a conductor, the electric charges move freely in response to the application of an electric field, whereas in an insulator or dielectric, the charges are fixed and not free to move. In practice, most materials, including human soft tissue, display some

characteristics of both insulators and conductors because they contain dipoles as well as charges that can move, but in a restricted manner. The dielectric properties of biological tissues are depending on its cellular structure.

The MMD generates a radiofrequency wave of 300MHz creating an alternating electromagnetic field in the underlying tissue. At this frequency the wave penetrates the interstitial space and cell membrane. Therefore, the wave interacts with both free water, predominantly found in the interstitium, and bound water in and outside the cell[117, 190]. Proteins inside and outside the cell have the capability to bind up to 0.7g of water per gram protein depending on architecture, charge and fold.

The frequency of 300MHz is extremely useful because the electromagnetic wave sent out by the coaxial open-ended probe causes bound water molecules to rotate like free water molecules. In other words, the radiofrequency generates an electromagnetic wave that moves through the tissue interacting equally with both free and bound water.

The wave will be partially absorbed by the tissue while the rest is reflected and detected by the outer spacer of the probe. The phase shift and change in magnitude of the detected wave will be analysed by the main unit[119]. For a soft tissue, the way water molecules behave to an applied electromagnetic field depends on the frequency. At low frequencies (lowkilohertz range), the tissue exhibits a so called 'alpha' dispersion of the electromagnetic wave. The magnitude and phase of the electromagnetic wave depends on polarization of water molecules near charged surfaces in the tissue and possibly the polarization of large membrane-bound water molecules in the tissue.

At radiofrequencies centred in the range 3KHz to 300MHz, the tissue shows 'beta' dispersion: the charging of cell membranes through the intracellular and extracellular media. Above the beta dispersion, the cell membranes have negligible impedance, and the current passes through both the extracellular and intracellular media. Therefore, the behaviour of biological tissues at these frequencies is largely determined by the electro-chemical behaviour of cells, its cellular structure as well as the extracellular fluid and the internal cellular materials.

At microwave frequencies (above 300MHz), the tissue exhibits a 'gamma' dispersion of the electromagnetic wave due to rotational relaxation of tissue water. In addition to these three major dispersions, other smaller dispersions occur due to rotational relaxation of bound water or tissue proteins, charging of membranes of intracellular organelles, and other effects. Due to the complex nature of biological materials, several of these processes are believed to co-exist at frequencies throughout the low kilohertz range and lower microwave band. Thus, the phase shift and difference in magnitude of the electromagnetic wave induced by the MMD is dominated by multiple dispersion patterns caused by both intercellular and extracellular water molecules[119].

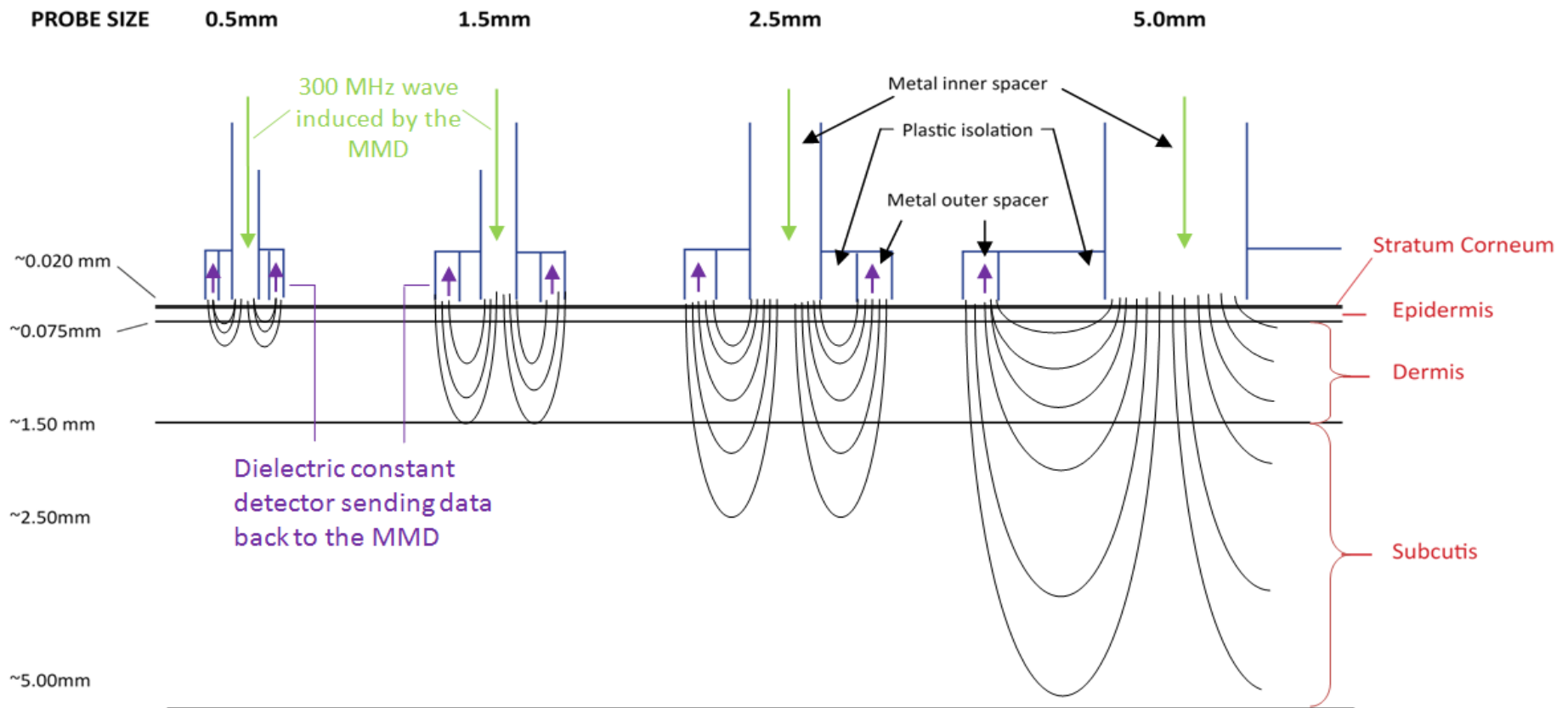
Although the penetration depth is primarily dependent on probe size, only frequencies above 100MHz possess the power to penetrate all layer of normal skin and reach into the subcutaneous fat[191, 192]. Lower frequencies are reflected by superficial structures and are significantly more influenced by the stratum corneum and top cell layers of the epidermis[192].

The effective thickness and structure of the skin varies with the anatomical site of the body. In order of depth below the skin surface, the skin of the forearm is composed of a poorly conducting stratum corneum (~0.020mm depth, 20% TWC), well conducting epidermis (~0.020-0.075mm depth, 70% TWC) and dermis (~0.075mm-1.5mm depth, 70% TWC) and the subcutis (~2-10mm depth, 10% TWC) [93, 192-203]. The effective tissue dielectric constant depends on the percentages of the electromagnetic wave reflected by the different layers. The water rich epidermis and dermis will increase the TDC reading, whereas the subcutaneous layer will lower the TDC reading.

The effective depth is determined by the designers of the MMD as the depth where the electric field has attenuated to 37% of its value at the surface[119]. In forearm skin the effective depth of electromagnetic wave generated by 0.5mm probe is believed to reach the superficial dermis. The 1.5mm probe reaches to the deep part of the dermis and 2.5mm probe to the complete dermis and superficial layers of the subcutaneous fat. A large portion of the electromagnetic wave coming from the 5.0mm probe is reflected by the subcutis. (*see fig. 4*)

In patients suffering from lymphedema, not only the interstitial water concentration in lymphedematous tissue changes, but often there is also a significant increase of dermal thickness, altering the ratio of waves reflected by the different layers [204].

Finally, If the device is calibrated correctly the assessment of a vacuum will result in a reading of 1 and pure water a reading of 78.5[119].



**Fig. 4 Schematic rendition of the interaction of the MMD probe and skin.** A 300MHz electromagnetic wave is induced by the MMD main unit and transported via a flexible cable to the inner spacer of the probe. Depending on the space between the inner transmitting spacer and the outer receiving spacer the electromagnetic waves travel deeper in the tissue interacting with variable percentages of underlying cell layers. The collected information at the outer spacer of the probe is send back to the MMD main unit to be analyzed. Please note that the actual depth of the epidermis, dermis and subcutis may vary (*see appendix 2,3 and 4*).

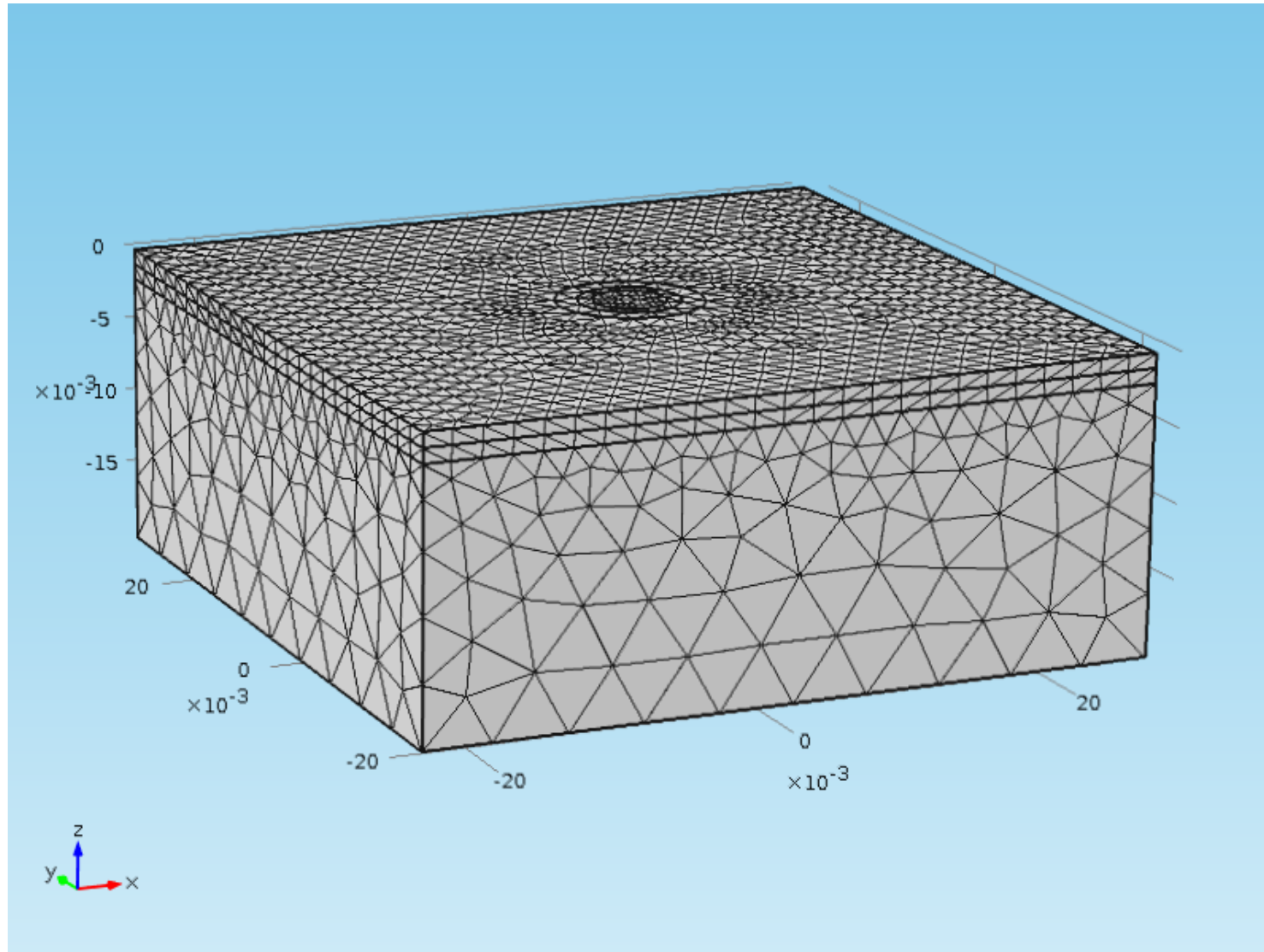


Fig. 5. A 3d model of the spread of the electromagnetic wave through the tissue

### **3.5 Self-reported measures of lymphedema and pain**

The participants completed a self-report questionnaire of their experience of lymphedema in ipsilateral the arm and breast (*attached in appendix 1*). Pain was rated on an 11-point numerical scale using anchors of 0 = no pain and 10 =worst pain imaginable. Additionally, discomfort, heaviness and the perception of increased size were measured on a 4-point scale using verbal descriptors of none, mild, moderate and severe. Patients were also asked if they had any skin changes in the arm or breast and to rate the success of their treatment on an 11-point numerical scale with anchors of 0 = not successful at all and 10 = completely successful. The women were also asked to give their opinion to the treatment they received in the lymphedema clinic.

### **3.6 Data analysis**

Statistical analysis was carried out using SPSS (Version 20, IBM, USA) and Prism (Version 5.0 GraphPad Inc, USA) with significance defined as  $P < 0.05$ . A repeated measure ANOVA (RM ANOVA) was used to identify significant differences in TDC values between the affected and control breast. The relationship of the TDC measurements for each probe and quadrant was examined using a 2 x 2 repeated measures ANOVA. Repeated measures general linear models (GLMs) were used for analysis of the depth and quadrant variation in breast TDC. The Huynh-Feldt correction was employed where Mauchly's test of sphericity was violated and the Bonferroni adjustment was used in post-hoc pairwise comparisons. Agreement between single and the average of triplicate measurements was evaluated using the Bland-Altman approach. Furthermore, Kendall's tau-b was used to correlate BMI and age with TDC.

## **4.0 RESULTS ARM LYMPHEDEMA MEASUREMENTS**

#### **4.1 Patients**

Twenty women with previous unilateral breast cancer-related surgery were recruited. The women presented with varying degrees of lymphedema and had attended the clinic for up to forty visits since their initial consultation.

Circumferential and TDC measurements of the arm were obtained in 18 of the 20 patients (age =  $61.3 \pm 9.9$  years; BMI =  $29.7 \pm 5.7$  kg/m<sup>2</sup>). The median amount of visits to the lymphedema clinic since their initial visit was 7.0 (95% confidence limits (CL): 4.7-14.6 visits) in a period varying from 2 months to 12 years (median 17.0 months, 95% CL: 11.6-50.5 months). The women were aged between 44 and 82 years (mean  $\pm$  SD =  $61.3 \pm 9.9$  years) and comprised of 8 BCT patients (age= $60.0 \pm 7.3$  years; BMI =  $31.4 \pm 1.8$  kg/m<sup>2</sup>) and 10 mastectomy patients (age = $62.3 \pm 11.8$  years; BMI =  $28.1 \pm 2.0$  kg/m<sup>2</sup>). Additional systemic treatment was administered in 17 patients: 7 patients received hormone therapy, 2 patients received chemotherapy and 8 patients received both. All but one patient was treated with postoperative radiotherapy. Body mass index (BMI) was calculated for each participant and, within the group, ranged from 20.9 to 39.3 kg/m<sup>2</sup> with a mean  $\pm$  SD of  $29.4 \pm 5.67$  kg/m<sup>2</sup>. There were no significant differences in age ( $t(16)=0.418$ ,  $p=0.637$ ) and BMI ( $t(15)=1.170$ ,  $p=0.260$ ) between the BCT and mastectomy groups. Details are summarized in table 3.

#### **4.2 Self-reported measures of lymphedema**

The patients' self-reported assessment of the lymphedema under treatment is presented in table 3. Out of the 18 patients tested, 72% (13 patients) reported some level of discomfort, 56% (10 patients) reported heaviness and 61% (11 patients) reported a perception of increased size of the ipsilateral arm following breast cancer surgery. There were no significant differences in the reported incidence for these parameters between the BCT and mastectomy groups. No patients reported any skin changes in the arm.

56% of the patients described pain in the affected arm (6 BCT and 4 mastectomy patients). When all patients were considered, numerical pain ratings in arm were not significantly different between the

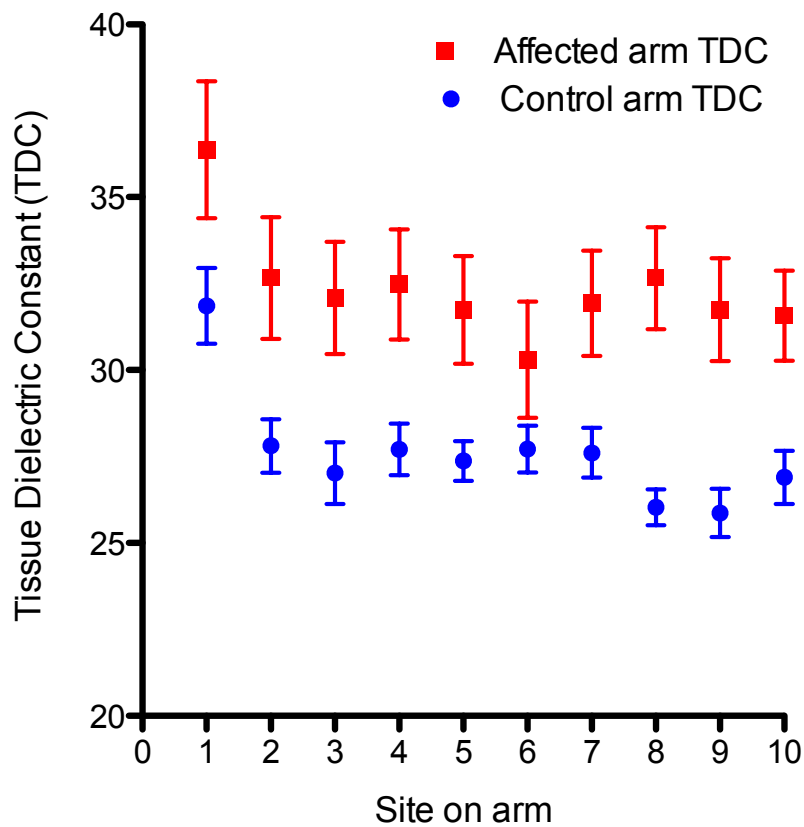
groups (Mann-Whitney U=62.5, p=0.312). Of those patients reporting pain, there were no significant differences found between the BCT and mastectomy groups for pain ratings of the arm. Where pain was reported, the mean numerical pain rating ( $\pm$ SD) was 4.5 ( $\pm$ 2.4) with a range from 1-8.

Subjective measure		All patients (n=18)		BCT (n=8)		Mastectomy (n=10)		p-value
Mean Age ( $\pm$ SD)	(years)	61.3	$\pm$ 9.86	60.0	$\pm$ 11.83	62.3	$\pm$ 7.25	0.637
Mean BMI ( $\pm$ SD)	(kg/m <sup>2</sup> )	29.7	$\pm$ 5.67	31.4	$\pm$ 5.13	28.2	$\pm$ 5.99	0.260
Number of visits (median + 95% CL)	(months)	7.0	2.5-12.5	9.0	4.0-15.0	3.0	2.0-12.0	0.228
Follow-up (median + 95% CL)	(months)	14.5	10-31.5	12.0	4.0-30.0	27.5	10.0-34.5	0.203
Lymphedema clinic attendance (median + 95% CL)	(months)	17.0	9.0-25.5	18.5	4.0-24.0	15.0	8.5-93.0	0.573
Chemotherapy	(n)	10	55.6 %	3	37.5 %	7	70.0 %	0.302
Homonotherapy	(n)	15	83.3 %	8	100.0 %	7	70.0 %	0.471
Hormone- + Chemotherapy	(n)	8	44.4 %	3	37.5 %	5	50.0 %	1.000
Radiotherapy	(n)	16	88.9%	7	87.5%	9	90%	n/a
Nodes removed	(n)	14	77.8%	6	75%	8	80%	n/a
Discomfort	None	6	33.3 %	4	50.0 %	2	20.0 %	0.433
	Mild	7	38.9 %	2	25.0 %	5	50.0 %	
	Moderate	4	22.2 %	1	12.5 %	3	30.0 %	
	Severe	1	5.6 %	1	12.5 %	0	0.0 %	
Perception of increased size	None	8	44.4 %	5	62.5 %	3	30.0 %	0.401
	Mild	5	27.8 %	1	12.5 %	4	40.0 %	
	Moderate	4	22.2 %	1	12.5 %	3	30.0 %	
	Severe	1	5.6 %	1	12.5 %	0	0.0 %	
Heaviness	None	9	50.0 %	5	62.5 %	4	40.0 %	0.647
	Mild	3	16.7 %	1	12.5 %	2	20.0 %	
	Moderate	4	22.2 %	0	0.0 %	4	40.0 %	
	Severe	2	11.1 %	2	25.0 %	0	0.0 %	

**Table 3: Patient characteristics and self-reported measures of lymphedema in the arm.** Values represent the number of patients reporting the discomfort, heaviness or the perception of increased size of the arm using a categorical rating scale of none, mild, moderate and severe. Abbreviations: BCT=Breast conserving therapy, SD=Standard Deviation, CL=Confidence Limits

### 4.3 TDC measurements the arm

TDC values at ten locations on the forearm were measured and the inter-subject variation is presented in Figure 6 for the affected and control arms. TDC values for the affected arms were found to be significantly higher than the control arms (RM ANOVA,  $F=14.79$ ,  $P=0.001$ ). The site of measurement along the proximal-distal axis was also found to be significant (position 1-10:  $F=9.94$ ,  $P<0.001$ , Huynh-Feldt corrected). Post-hoc tests revealed that site 1 (positioned close to the wrist) had significantly higher TDC values than all other sites. In contrast, combinations of all other sites from 2-10 did not show any significant differences.



**Fig. 6: Average proximal distal TDC trend** for all patients. Measurements taken at the ventral side of the arm at 4cm intervals, with site 1 positioned at 19cm from the base of the nail of the third finger.

Therefore the ANOVA was repeated with site 1 excluded and, whilst the differences in the arm tested were still significant as would be expected (affected > control,  $F=15.07$ ,  $P=0.001$ ), the measurement position along the arm was no longer found to be significant (position 2-10:  $F=15.1$ ,  $P=0.35$ ).

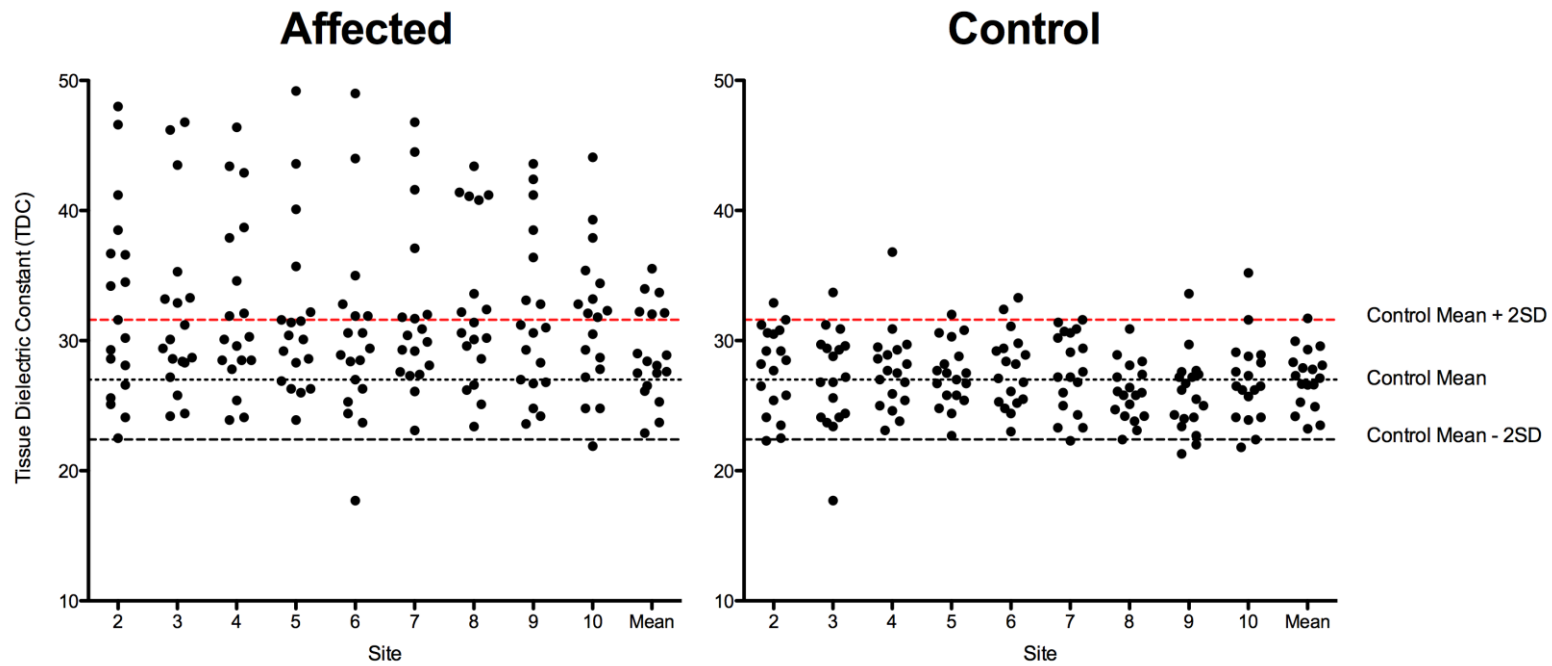
Consequently, a mean TDC measure for sites 2-10 (mean TDC2-10) was used for further analysis.

Absolute TDC measurements for each individual and the mean TDC2-10 measure for the affected and control arms are shown in Figure 7. The mean TDC2-10 for the affected arms were found to be significantly higher and demonstrated more variance than for the control arms (affected: mean TDC2-10  $\pm$  SD =  $31.6 \pm 5.9$ , control: mean TDC2-10  $\pm$  SD =  $27.0 \pm 2.3$ , paired-t:  $t=3.882$ ,  $P=0.001$ ).

The mean TDC value for the affected arms was positively correlated with age of the patient (Kendall's tau=0.475,  $P=0.006$ ) but not their BMI (Kendall's tau=-0.162,  $p=0.365$ ). The equivalent correlation with age for the control arm was also close to significance (Kendall's tau=0.317,  $P=0.068$ ) but, similarly, no correlation was found with BMI (Kendall's tau=0.059,  $p=0.742$ ). The ratio of the affected and control means also displayed the same trend, with age being positively correlated (Kendall's tau=0.343,  $P=0.048$ ) but BMI showing no correlation (Kendall's tau=-0.147,  $P=0.41$ ).

#### **4.4 Volume measurements**

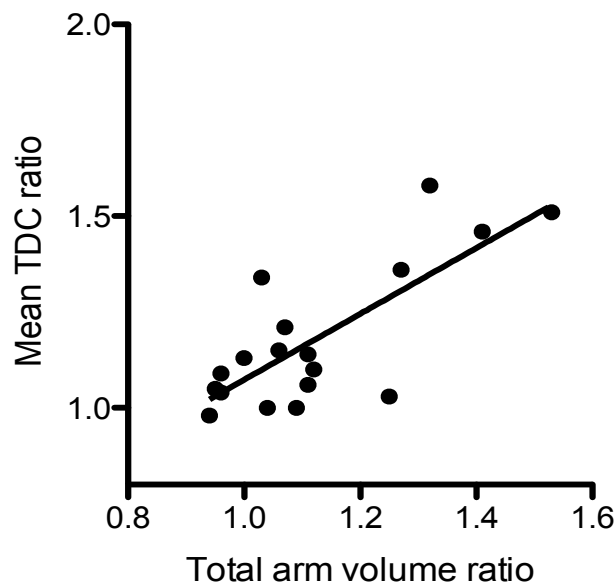
The mean arm volume was found to be significantly higher for affected arms (mean  $\pm$  SD =  $2302 \pm 588$  ml, range=1430-3477) than control arms (mean  $\pm$  SD =  $2023 \pm 463$  ml, range=1155-2629 ml, paired-t:  $t=3.28$ ,  $P=0.004$ ). The ratio of affected to control for the volume measurements ranged from 0.89 to 1.68 with a mean and standard deviation of  $1.15 \pm 0.21$ . Four patients scored a ratio less than 1 (median  $0.95 \pm 0.03$ , range 0.89-0.96) indicating a higher volume of the control arm (*See fig. 8*). As with the TDC measurements, a significant positive correlation was found between age and arm volume ratio (Kendall's tau=0.502,  $p=0.004$ ) but not BMI (Kendall's tau = -0.044,  $p=0.805$ ).



**Fig. 7: Variation of the tissue dielectric constant (TDC) values with position on the forearm for the affected and control arms.** The TDC values across sites 2-10 for each patient are also shown. The dotted lines plotted on each graph detail the mean and two standard deviation ( $\pm 2SD$ ) limits for the control arms. Measurement sites on the arm were separated by 4cm with site 2 is positioned 23cm from the base of the nail of the third finger.

#### 4.5 Comparison of arm TDC, volumetric and self-reported measures

The ratio of the TDC between affected and control arm ranged from 0.98 to 1.58, with only one patient with a ratio <1. The mean TDC ( $1.18 \pm 0.19$ ) measurements for the affected and control arms was positively correlated with the total arm volume ratio as shown in Figure 8 (Kendall's tau = 0.830,  $p < 0.001$ ). As we showed previously that both volumetric and TDC ratios were correlated with age, a partial correlation controlling for age, also resulted in a strong positive correlation between volume and TDC ratios (Pearson's  $R = 0.677$ ,  $p = 0.003$ ).



**Figure 8: comparison of volume ratio and TDC ratio values** between affected and control arm.

When the type of surgery was investigated, mastectomy patients were found to have higher TDC ratios than BCT patients ( $p = 0.009$ ) but there were no significant differences observed in the volume ratio (see table 4). The ratio of the TDC values was also found to be significantly higher for patients with pain ( $p = 0.014$ ) and discomfort ( $p = 0.0041$ ) but not for heaviness or perception of increased size. No significant differences were found in volume ratio for patients with/without pain, discomfort, heaviness or perception of increased size. In contrast to the TDC ratio between affected and control arm none of the subjective self-report measures correlates significantly with the absolute TDC value of the affected arm. The results are shown in table 5.

Variable		Number of patients	TDC ratio			Volume ratio		
			Mean rank	Mann-Whitney U	Sig.	Mean rank	Mann-Whitney U	Sig.
Surgery type	BCT	8	5.88	69	0.009*	7.25	58	0.122
	Mastectomy	10	12.40			11.30		
Pain	Yes	9	12.56	68	0.014*	11.22	56	0.190
	No	9	6.44			7.78		
Discomfort	Yes	12	11.33	58	0.041*	10.83	52	0.151
	No	6	5.83			6.83		
Heaviness	Yes	9	11.11	55	0.222	11.56	59	0.113
	No	9	7.89			7.44		
Increased size	Yes	10	11.50	60	0.083	11.40	59	0.101
	No	8	7.00			7.12		

**Table 4: Comparison of the self-reported measures of arm lymphedema with the objective arm volume and TDC ratios.** \* p < 0.05 considered significant

Variable		Number of patients	Absolute TDC affected arm			Absolute Volume affected arm		
			Mean rank	Mann-Whitney U	Sig.	Mean rank	Mann-Whitney U	Sig.
Surgery type	BCT	8	7.50	56	0.173	9.12	43	0.829
	Mastectomy	10	11.10			9.80		
Pain	Yes	9	11.33	57	0.161	9.56	41	1.000
	No	9	7.67			9.44		
Discomfort	Yes	12	10.75	51	0.180	10.08	43	0.553
	No	6	7.00			8.33		
Heaviness	Yes	9	10.67	51	0.387	10.89	53	0.297
	No	9	8.33			8.11		
Increased size	Yes	10	11.00	55	0.203	11.70	62	0.054
	No	8	7.62			6.75		

**Table 5: Comparison of the self-reported measures of arm lymphedema with the objective arm volume and TDC measures.** In contrast to the TDC ratio (shown in table 4) none of the subjective self-report measures correlates significantly with the absolute TDC value of the affected arm.

## **5.0 RESULTS BREAST LYMPHEDEMA MEASUREMENTS**

## 5.1 Patients

Twenty women with previous unilateral breast cancer-related surgery were recruited. The women presented with varying degrees of lymphedema and had attended the clinic for up to forty visits since their initial consultation (median: 9.0, 95% confidence limits (CL): 5.5-16.7 visits) over two months to twelve years (median 18.5 months, 95% CL: 15.1-50.6 months). The women were aged between 44 and 82 years (mean  $\pm$  SD = 61.3  $\pm$  9.6 years) and comprised of 10 lumpectomy patients (60.9  $\pm$  7.3 years) and 10 mastectomy patients (62.3  $\pm$  11.8 years). One woman who had previously had a lumpectomy followed by a later mastectomy was assigned to the mastectomy group. The women had a body mass index (BMI) from 18.4 to 39.3 and mean  $\pm$  SD of 29.4  $\pm$  6.13. There were no significant differences in age and BMI between the lumpectomy and mastectomy groups. Patient characteristics are summarized in table 6.

## 5.2 Self-reported measures of lymphoedema

The patients' self-reported assessment of the lymphoedema under treatment is presented in table 6. Out of the 10 lumpectomy and 10 mastectomy patients tested, 13 patients (65%) reported some level of discomfort, 10 patients (50%) reported heaviness and 11 patients (55%) reported a perception of increased size of the ipsilateral breast following breast cancer surgery. 70% of patients reported discomfort in the breast on the ipsilateral side, with the BCT patients reporting significantly more discomfort than the mastectomy patients (Mann-Whitney U=19.0, Z=-2.463, P=0.014). There was a significant difference for increased heaviness and size perception between the BCT and mastectomy group. Seven BCT patients reported heaviness and eight reported a perception of increased size. Interestingly, one mastectomy patients also reported heaviness and two reported the perception of increased size on the operated side. No patients reported any skin changes in the arm or breast.

65% (9 BCT and 4 mastectomy patients) reported pain in the breast. When all patients were considered, numerical pain ratings in the breast were found to be significantly higher in the BCT

Subjective measure		All patients (n=20)		BCT (n=10)		Mastectomy (n=10)		p- value
Mean Age ( $\pm$ SD)	(years)	61.3	$\pm$ 9.56	60.3	$\pm$ 7.12	62.3	$\pm$ 11.83	0.631
Mean BMI ( $\pm$ SD)	(kg/m <sup>2</sup> )	29.4	$\pm$ 6.13	30.5	$\pm$ 6.36	28.2	$\pm$ 5.99	0.422
Number of visits (median + 95% CL)	(months)	9.0	3.0-13.0	9.0	4.0-19.0	3.0	2.0-12.0	0.156
Follow-up (median + 95% CL)	(months)	14.5	9.0-30.5	12.0	4.0-27.0	27.5	10.0-33.0	0.143
Lymphoedema clinic attendance (median + 95% CL)	(months)	18.5	11.5-30.0	21.0	7.0-36.4	15.0	9.5-84.0	0.853
Chemotherapy	(n)	10	50.0%	3	30.0 %	7	70.0 %	0.153
Homonotherapy	(n)	15	75.0 %	8	80.0 %	7	70.0 %	0.471
Hormone + Chemotherapy	(n)	8	40.0%	3	30.0%	5	50.0 %	0.673
Radiotherapy	(n)	17	85.0%	8	80.0%	9	90%	n/a
Nodes removed	(n)	16	80.0%	8	80.0%	8	80%	1.000
Discomfort	None	6	30.0 %	1	10.0 %	5	50.0 %	0.014*
	Mild	7	35.0 %	3	30.0 %	4	40.0 %	
	Moderate	6	30.0 %	5	50.0 %	1	10.0 %	
	Severe	1	5.0 %	1	10.0 %	0	0.0 %	
Perception of increased size	None	10	50.0 %	2	20.0 %	8	80.0 %	0.013*
	Mild	5	25.0 %	4	40.0 %	1	10.0 %	
	Moderate	4	20.0 %	3	30.0 %	1	10.0 %	
	Severe	1	5.0 %	1	10.0 %	0	0.0 %	
Heaviness	None	12	60.0 %	3	30.0 %	9	90.0 %	0.013*
	Mild	3	30.0 %	3	30.0 %	0	0.0 %	
	Moderate	4	20.0 %	3	30.0 %	1	10.0 %	
	Severe	1	10.0 %	1	10.0 %	0	0.0 %	

**Table 6: Patient characteristics and self-reported measures of lymphoedema in the breast.** Values represent the number of patients reporting the discomfort, heaviness or the perception of increased size of the breast using a categorical rating scale of none, mild, moderate and severe. For the subjective self-report rating between the BCT and mastectomy group the Mann-Whitney U test was employed. Abbreviations: BCT=Breast conserving therapy, SD=Standard Deviation, CL=Confidence Limits. \*P<0.05 consider significant.

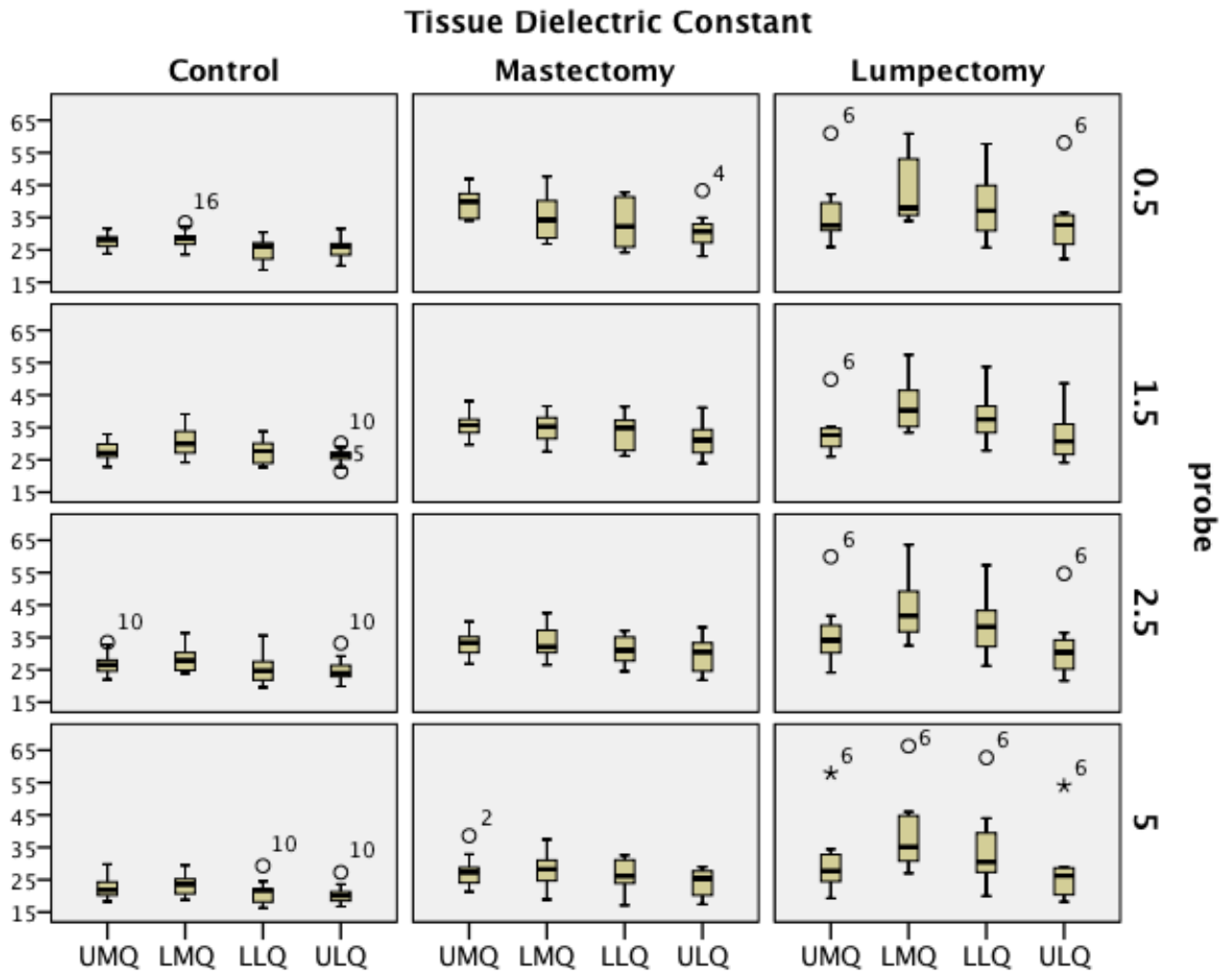
group than the mastectomy group ( $U=23.5$ ,  $Z=-2.065$ ,  $p<0.05$ ) whereas numerical pain ratings in arm were not significantly different between the groups ( $U=62.5$ ,  $Z=1.011$ ,  $p=0.312$ ). However, if only the patients reporting pain were considered, there were no significant differences found between the BCT and mastectomy groups for the pain ratings in the breast. Of the patients that reported pain, the mean numerical rating ( $\pm$ SD) was 3.5 ( $\pm$  2.9) for pain located in the breast.

### **5.3 Breast TDC measurements**

The variation of the TDC data for each quadrant and probe for the control, post-mastectomy and post-BCT breasts are presented in Figure 9 and table 7.

In the mastectomy group the average TDC of the affected breast was significantly higher for all quadrants when compared to the control breast at all measurement depths with the exception of the lower medial quadrant (LMQ) at a depth of 1.5mm.

In the BCT group the average TDC of the affected breast was higher in all quadrants at the depths of 1.5, 2.5 and 5.0mm with the exception of the upper lateral quadrant (ULQ) at 5.0mm. The results for measurements taken in the upper medial quadrant (UMQ) showed only a significant difference between BCT and control breast at a depth of 2.5mm.



**Fig. 9: Tissue dielectric constant (TDC) values** for each quadrant of the breast. Quadrants are defined as: upper medial quadrant (UMQ), lower medial quadrant (LMQ), lower lateral quadrant (LLQ) and upper lateral quadrant (ULQ). Data is presented for probes with diameter of 0.5, 1.5, 2.5 and 5.0mm that corresponds to measurements at different depth within the tissue.

		Mastectomy group N=10						BCT Group N=8					
Quadrant	Probe	Control Breast		Affected Breast		t	p-value	Control Breast		Affected Breast		t	p-value
		Mean	Std.	Mean	Std.			Mean	Std.	Mean	Std.		
UMQ	0.5 mm	27.7	1.4	39.4	4.8	-8.00	<0.001*	27.4	2.9	36.6	11.0	-2.10	0.074
	1.5 mm	27.6	2.2	35.9	4.1	-5.63	<0.001*	27.1	3.0	33.6	7.3	-2.28	0.057
	2.5 mm	26.8	3.2	33.2	4.0	-5.62	<0.001*	26.5	3.4	36.3	10.8	-2.70	0.031*
	5.0 mm	22.3	3.3	27.5	5.2	-3.35	0.009*	22.6	2.6	30.1	11.9	-2.07	0.078
LMQ	0.5 mm	28.7	2.7	35.2	7.0	-2.87	0.019*	27.4	2.6	43.5	10.4	-4.28	0.004*
	1.5 mm	31.4	4.1	34.6	4.6	-1.65	0.134	29.6	3.9	41.9	8.1	-4.58	0.003*
	2.5 mm	28.5	4.0	33.6	5.0	-2.68	0.025*	27.4	2.9	43.9	10.0	-5.91	0.001*
	5.0 mm	23.4	3.2	27.8	5.4	-2.80	0.021*	23.1	3.4	39.4	12.7	-4.35	0.003*
LLQ	0.5 mm	25.4	3.1	33.4	7.8	-2.85	0.019*	24.8	3.4	38.6	10.5	-4.09	0.005*
	1.5 mm	28.3	3.6	33.8	5.3	-3.03	0.014*	27.0	3.5	38.3	7.8	-5.66	0.001*
	2.5 mm	25.4	4.5	31.3	4.5	-3.28	0.010*	24.7	2.8	38.9	9.7	-5.15	0.001*
	5.0 mm	21.3	3.4	26.3	4.8	-3.46	0.007*	20.4	3.1	34.7	13.3	-3.30	0.013*
ULQ	0.5 mm	26.6	2.7	31.1	5.4	-2.46	0.036*	24.6	3.7	33.8	11.0	-2.44	0.045*
	1.5 mm	27.1	1.5	31.0	5.1	-2.33	0.045*	25.0	2.4	32.4	7.9	-3.19	0.015*
	2.5 mm	25.5	3.3	29.6	5.3	-2.92	0.017*	24.0	2.8	32.0	10.4	-2.64	0.033*
	5.0 mm	20.6	2.8	24.4	4.0	-4.35	0.002*	20.0	2.6	27.8	11.3	-2.14	0.070

**Table 7: Mean tissue dielectric constant (TDC) values** for each quadrant of the breast. Quadrants are defined as: upper medial quadrant (UMQ), lower medial quadrant (LMQ), lower lateral quadrant (LLQ) and upper lateral quadrant (ULQ). Data is presented for probes with diameter of 0.5, 1.5, 2.5 and 5.0mm that corresponds to measurements at different depth within the tissue. \*p<0.05 is considered significant.

### **5.3.1 TDC measurements of the control breasts**

The relationship of the TDC measurements for each probe and quadrant was examined using a 2 x 2 repeated measures ANOVA. The TDC data demonstrated a significant effect of both probe ( $F=91.32$ ,  $P<0.001$ ) and quadrant ( $F=18.724$ ,  $P<0.001$ ). There was also an interaction between probe and quadrant ( $F=3.840$ ,  $P<0.001$ ). Post-hoc tests for probe revealed that the TDC measurements from all the probes were significantly different from each other at  $P<0.05$  level except for the values recorded with the 0.5mm and 2.5mm probes (mean difference=0.538,  $P=1.00$ ).

Post-hoc tests for quadrant revealed that medial and lateral quadrants for a given inferior or superior location (i.e. UMQ vs. ULQ, LMQ vs. LLQ) always had significantly higher TDC values in the medial quadrant when compared with the lateral ( $P<0.05$ ). A further 4x2x2 repeated measures ANOVA was performed to look for additional factors of 1) medial vs. lateral and 2) upper vs. lower and 3) any interactions between these factors and the probe used. Medial quadrants were found to have significantly higher TDC values than lateral quadrants (mean difference=2.282,  $F=72.142$ ,  $P<0.001$ ). Upper quadrants were found to have significantly lower TDC values than the lower quadrants (mean difference=-0.972,  $F=7.338$ ,  $P=0.015$ ).

Furthermore, an interaction between probe and the upper vs. lower factor was found ( $F=9.235$ ,  $P<0.001$ ) indicating that the behaviour of the TDC measurements with depth was different between the upper and lower quadrants.

### **5.3.2. TDC measurements of the post-mastectomy breasts**

The TDC data for the post-mastectomy breasts demonstrated a significant effect of probe ( $F=51.40$ ,  $P<0.001$ ) and quadrant ( $F=6.71$ ,  $P=0.002$ ). There was also an interaction between probe and quadrant ( $F=2.56$ ,  $P=0.017$ , Huynh-Feldt corrected). Post-hoc tests for probe revealed that the TDC measurements from the 1.5mm probe were not significantly different from the 0.5mm (mean difference=-1.269,  $P=0.989$ ) or 2.5 mm (mean difference=1.521,  $P=0.061$ ) probes. All other probe combinations were significantly different from each other at  $P<0.05$  or lower significance levels. As

with the control breasts, TDC measurements in the medial quadrants were found to be significantly higher than those of the lateral quadrants (mean difference = 3.245,  $F=17.132$ ,  $P=0.003$ ). However, in contrast to the control breasts, TDC measurements in the upper quadrants displayed the same trend but were not significantly lower when compared to the lower quadrants (mean difference =  $-0.455$ ,  $F=0.262$ ,  $P=0.621$ ).

### **5.3.3. TDC measurements of the post-‘breast conserving therapy’ breast**

The TDC data for the post-lumpectomy breasts demonstrated a significant effect of probe ( $F=9.26$ ,  $P=0.004$ , Huynh-Feldt corrected) and quadrant ( $F=17.57$ ,  $P<0.001$ ). However, in contrast to the control and mastectomy breasts, the interaction between probe and quadrant was not found to be significant ( $F=0.792$ ,  $P=0.624$ ). Post-hoc tests for probe revealed that the TDC measurements from 5.0mm probe were significantly different from the 0.5mm (mean difference= $-5.916$ ,  $P=0.01$ ) and 2.5 mm (mean difference= $-4.778$ ,  $P=0.003$ ) probes. All other probe combinations were not found to have significantly different TDC values. As reported previously for the control and mastectomy breasts, TDC measurements in the medial quadrants were significantly higher than those of the lateral quadrants (mean difference = 3.355,  $F=15.575$ ,  $P=0.004$ ).

Additionally, as for the control breasts, TDC measurements in the upper quadrants were significantly lower when compared to the lower quadrants (mean difference =  $-6.538$ ,  $F=25.299$ ,  $P=0.001$ ).

No correlation of TDC values with age or BMI in any quadrant for affected or control breasts.

### **5.4 Comparison of breast TDC and self-reported measures**

The mean TDC ratio between the affected and control breast was significantly higher in the lower medial quadrant (LMQ) of the breast at the measurement depths of 1.5, 2.5 and 5.0mm in the BCT group. The same trend can be spotted for the lateral lower quadrant (LLQ), but no differences are seen in any of the upper quadrants (*See table 8*).

The patients reporting pain showed a trend of higher TDC ratios in all four quadrants at all depths, (with the exception of the UMQ at a depth of 1.5mm,) becoming significant at the two deeper measurements in the LLQ. The mean TDC ratio for patients reporting discomfort in the breast was significantly higher in the lateral quadrants at the depth of 2.5mm, the ULQ at a depth of 0.5mm and the LLQ at a depth of 5.0mm. Although the mean TDC ratio for patients reporting discomfort showed the same trend for the medial quadrants, especially at a depth of 2.5mm, no significant differences were seen with the patients who did not experience discomfort (*See table 8*).

There was no correlation between self experienced heaviness of the breast and mean TDC ratio. Significantly higher mean TDC ratios were found in the LLQ at all depths and the LMQ at depths of 1.5 and 2.5mm in the patients that reported a sensation of increased size. The same trend could be spotted in the other measurement depth in the LMQ, albeit not significant. The upper quadrants showed no correlation at all between perceived heaviness and mean TDC ratios (*See table 8*).

Measurement depth 0.5mm		Number of patients	UMQ			LMQ			LLQ			ULQ		
			Mean rank	Mann-Whitney U	Sig.	Mean rank	Mann-Whitney U	Sig.	Mean rank	Mann-Whitney U	Sig.	Mean rank	Mann-Whitney U	Sig.
Surgery type	BCT	8	7.75	54	0.237	12.25	18	0.051	11.50	24	0.155	11.50	24	0.173
	Mastectomy	10	10.9			7.3			7.90			7.90		
Pain	Yes	11	9.91	43	0.724	10.27	47	0.441	11.00	18	0.135	11.00	55	0.151
	No	7	8.86			8.29			7.14			7.14		
Discomfort	Yes	12	8.17	44	0.494	10.42	47	0.303	11.08	55	0.075	12.03	67	<0.01*
	No	6	10.17			7.67			6.33			4.33		
Heaviness	Yes	6	7.00	21	0.180	10.33	41	0.640	9.33	35	0.925	12.00	51	0.180
	No	12	10.75			9.08			9.58			8.25		
Increased size	Yes	8	9.62	41	1.000	12.25	62	0.051	12.38	63	0.043*	11.75	58	0.122
	No	10	9.40			7.30			7.20			7.70		
Measurement depth 1.5mm		Number of patients	UMQ			LMQ			LLQ			ULQ		
			Mean rank	Mann-Whitney U	Sig.	Mean rank	Mann-Whitney U	Sig.	Mean rank	Mann-Whitney U	Sig.	Mean rank	Mann-Whitney U	Sig.
Surgery type	BCT	8	7.75	54	0.237	12.38	17	0.043*	12.12	19	0.068	11.25	26	0.237
	Mastectomy	10	10.90			7.20			7.40			8.10		
Pain	Yes	11	9.36	37	0.930	10.91	54	0.179	11.36	59	0.069	10.73	52	0.246
	No	7	9.71			7.29			6.57			7.57		
Discomfort	Yes	12	10.33	46	0.385	10.75	51	0.180	11.17	56	0.067	10.92	53	0.125
	No	6	7.83			7.00			6.17			6.67		
Heaviness	Yes	6	7.17	22	0.213	10.33	41	0.682	10.33	41	0.682	10.33	41	0.682
	No	12	10.67			9.08			9.08			9.08		
Increased size	Yes	8	9.30	42	0.897	12.62	65	0.027*	13.00	68	0.012*	10.00	44	0.762
	No	10	9.75			7.00			6.70			9.10		

Measurement depth 2.5mm		Number of patients	UMQ			LMQ			LLQ			ULQ		
			Mean rank	Mann- Whitney U	Sig.	Mean rank	Mann- Whitney U	Sig.	Mean rank	Mann- Whitney U	Sig.	Mean rank	Mann- Whitney U	Sig.
Surgery type	BCT	8	9.75	38	0.897	13.38	9	<0.01*	12.25	18	0.054	11.00	28	0.315
	Mastectomy	10	9.30			6.40			7.30			8.30		
Pain	Yes	11	11.27	58	0.085	11.00	55	0.151	11.64	62	0.035*	11.18	57	0.104
	No	7	6.71			7.14			6.14			6.86		
Discomfort	Yes	12	11.25	57	0.053	10.83	52	0.151	11.67	62	0.014*	11.33	58	0.042*
	No	6	6.00			6.83			5.17			5.83		
Heaviness	Yes	6	8.00	27	0.437	11.33	47	0.336	10.33	41	0.682	10.17	40	0.750
	No	12	10.25			8.58			9.08			9.17		
Increased size	Yes	8	11.25	54	0.237	12.62	65	0.027*	13.00	68	0.011*	9.75	42	0.897
	No	10	8.10			7.00			6.70			9.30		
Measurement depth 5.0mm		Number of patients	UMQ			LMQ			LLQ			ULQ		
			Mean rank	Mann- Whitney U	Sig.	Mean rank	Mann- Whitney U	Sig.	Mean rank	Mann- Whitney U	Sig.	Mean rank	Mann- Whitney U	Sig.
Surgery type	BCT	8	9.75	38	0.897	13.25	10	<0.01*	12.25	18	0.055	10.75	30	0.408
	Mastectomy	10	9.30			6.50			7.30			8.50		
Pain	Yes	11	11.73	63	0.027*	10.36	48	0.452	11.55	61	0.044*	10.82	53	0.211
	No	7	6.00			8.14			6.29			7.43		
Discomfort	Yes	12	10.42	47	0.336	10.67	50	0.213	11.42	59	0.032*	11.25	57	0.053
	No	6	7.67			7.17			5.67			6.00		
Heaviness	Yes	6	8.00	27	0.437	10.50	42	0.616	9.67	37	1.000	9.50	36	1.000
	No	12	10.25			9.00			9.42			9.50		
Increased size	Yes	8	9.75	42	0.897	11.88	59	0.101	12.75	66	0.021*	9.38	39	0.965
	No	10	9.30			7.60			6.9			9.60		

**Table 8.** Mean TDC ratio per measurement depth and quadrant related to operation type and subjective measures. \*p<0.05 is considered significant.

## **6.0 DISCUSSION**

## 6.1 Background and method consideration

The number of patients diagnosed with breast cancer every year is increasing[18]. Fortunately, developments in treatment have led to significantly increased survival rates[19] and less breast cancer related morbidity. Nevertheless, a significant group of patients still experience a diminished quality of life and suffer from complications thought to be caused by lymphedema. Lymphedema is a chronic condition characterized by an accumulation of proteins and fluid in the interstitial space of the affected tissue. Although it is clear that the increase of interstitial fluid is caused by the destructive effect of breast cancer treatment on the lymphatic system, it remains unclear to what extent. Incidence rates, relative risk ratios and numbers on the effect of different treatment modalities vary significantly because there is no general consensus on the definition of lymphoedema. Many different methods to assess lymphoedema have been developed over the past decades, but they all have their shortcomings. In order to understand the different aspects of the condition an objective quantitative assessment method is necessary to develop an unambiguous definition of lymphoedema.

Volume measurements are often regarded as the most reliable method of diagnosing BCRL[83]. However, the required comparison between affected and control arms means this cannot be used to assess bilateral lymphedema. Furthermore, volumetric assessment has yet to be standardised across centres and is insensitive to small changes and therefore has limited capability in detecting early stage BCRL. We have described the use of a novel technique based on the assessment of the tissue dielectric constant, which is directly related to the tissue water content (TWC) and therefore theoretically linked to the degree of lymphoedema in the tissue.

The effective thickness and structure of the skin varies with the anatomical site of the body. All our measurements were taken on the dorsal aspect of the arm. In order of depth below the skin surface, the skin of the forearm is composed of a poorly conducting stratum corneum (~0.020mm depth, 20% TWC), well conducting epidermis (~0.020-0.075mm depth, 70% TWC) and dermis (~0.075mm-1.5mm depth, 70% TWC) and the subcutis (~2-10mm depth, 10% TWC) [93, 192-203]. Appendix 2 gives an

overview of the thickness of different components of the arm skin as found in literature. TDC readings using the MMD depend mainly on three factors: the water concentration of the tissue, the skin thickness, particularly the thickness of the dermis, and the effective depth of the probe. In other words, the TDC reading depends on the percentage of electromagnetic wave that are reflected by the high water content dermis in contrast to the low water content subcutaneous fat. Not only the interstitial water concentration in lymphedematous tissue changes, but often there is also a significant increase of dermal thickness, altering the ratio of waves reflected by the different layers (*see appendix 3*) [204].

Since lymphedema normally presents in the deep dermis and superficial subcutis[204-206], we chose to use the 2.5mm probe as it has been proven previously to be the most effective depth in detecting changes in TWC in BCRL in the arm[121, 123, 207]. Due to the fact that there is limited data available regarding the interaction of electromagnetic waves and superficial breast tissue, we investigated all four probe sizes in each quadrant of the breast.

### **6.1.1. Volume measurement of the arm and TDC**

The mean volume calculated from circumferential measurements was significantly higher (275ml) for the affected side compared to the control arm, correlating with mean TDC values found in this research (*fig. 5*).

Absolute total arm volume differed significantly between patients, but in most patients the affected arm was significantly bigger than the control. However, 4 patients showed a volume ratio less than one, indicating a smaller affected arm compared to the control, despite feeling subjective sensations often associated with lymphedema in the affected arm. Interestingly enough, the MMD was capable to detect a higher TWC in the relatively smaller affected arm in three of these four patients, supporting the claim that the TDC assessment method can detect small changes better than the circumference method.

Most research reports similar total volumes for both the affected and control arm [83, 112, 208]. However, some report higher total arm volumes in a patient group with comparable BMI[121, 147] or lower BMI[209]. Whereas the affected arm volume can differ depending on the severity of the lymphedema, the mean control arm volume at a given BMI should be more or less similar. Without mutual agreement on positions of measurement and calculation method the arm circumference will never be an adequate method to compare BRCL assessments between different clinics.

Despite the heterogeneity of the patients in our population, TDC measurements in the affected arms were found to be higher than in the control arms in all but one patient. The absolute values were higher than those presented in earlier studies[207], presumably because with measured the dorsal rather than the ventral forearm skin. The skin on the dorsal aspect of the arm has a higher mean skin thickness (~1.5mm versus ~1.0mm, see appendix 2) and thus an increased contribution to the TDC from the water-rich epidermal and dermal layers.

The two significant outliers with reduced TDC values, which can be seen in Figure 4, are from the same patient and demonstrate the importance of maintaining similar pressure on the skin surface each time when obtaining TDC values.

### 6.1.2 Localization of arm TDC measurement

After excluding site 1 (positioned at the wrist) the results show that there was no significant difference in the TDC between any of the other sites on the arm in both the affected and control arm. Although measurements on the affected arm compared to the control showed a greater variance, all TDC values measured on the sites of the affected arm were significantly higher than TDC's measured on the control arm. This suggests that single TDC measurement can be sufficient at any site along the dorsal aspect of the arm, avoiding the skeletal parts in the wrist and elbow. This supports the previous findings by Mayrovitz et al.[207]. The bone structure of the elbow may also be responsible for the relatively small difference between control and affected arm at site 6 (*see fig. 3*). In contrast to the TDC method, an agreed number circumference measurements of precisely located sites are necessary to compute reproducible and accurate arm volume estimations.

However, several groups hypothesize that that lymphatic pathways of the limb do not flow in an absolute linear, progressive fashion and that segmental variations do exist [71, 210, 211]. They suggest that initial clinical presentation of lymphedema will show as fluid retention local to the muscle compartments predominantly in the pericubital region[71]. Therefore hypothetically to detect BCRL in the absolute beginning of the condition the location of the TDC measurement does matter. Further research is needed to provide evidence for this theory. Nevertheless, in patients with established or clinical evident lymphedema the location of the TCD measurement is irrelevant.

Lymphedema assessment using a single site TDC measurement method would lead to a significant time saving in clinic since fewer sites need to be assessed and their exact locations do not need to be measured.

### 6.1.3 Arm TDC and age

We found that older age was significantly correlated with a higher absolute TDC value in the affected arm. Additionally, we found that the TDC values for control arms demonstrated a non-significant positive correlation with age. In agreement with age being a risk factor for BCRL, we found that older patients had higher TDC ratio when comparing the affected arm to the control arm[212].

The only other research relating age and TDC in a group of healthy women found similar results for measurement depths of 0.5mm and 1.5mm but not 2.5mm [123], although the absolute TDC numbers were lower. A possible explanation for this dissimilarity is that the thinner ventral side of the arm was used for the measurements. At the ventral side of the arm in older patients a relatively large proportion of the electromagnetic pulse is reflected by the low water content subcutaneous fat tissue, lowering the TDC value.

This is unexpected because of higher TWC values at older age, skin thickness at the extremities decreases with increasing age. Thinning of the skin with aging results as a consequence of a decrease in dermal collagen synthesis[213, 214]. The rate in which this decrease occurs is equal for the ventral and dorsal side of the forearm, with an absolute higher thickness of the dorsal side[215].

Although there is disagreement for how long, many agree on a period in life in which the skin thickness stays level. Some studies show an invariable skin thickness till the age of 40 for women (in contrast to the constant minor decrease in men), after which the skin thickness start to decrease[216]. De Rigal et al.[217] reported a constant skin thickness between the second and seventh decade of life, but thinning begins after the seventh decade for the dorsal aspect, and eight decade for the ventral aspect. Escoffier et al. reported no significant variation in skin thickness between 15 and 65 years old individuals in a study assessing 54 men and 64 with ultrasound.

However, after the age of 65 skin thickness decreases significantly[218]. Additionally, it is believed that photo-exposed skin thickness decreases significantly more and more quickly than skin at protected body areas[215, 219], with the exception of the stratum corneum. The stratum corneum does not change[200, 220] or even increases with age (especially at photo exposed sites)[195, 221,

222]. Since the stratum corneum and the rest of the epidermis are relatively thin, the major decrease in skin thickness is related to the degeneration of the collagen network in the underlying dermis[194, 223].

The higher TDC values in older patients cannot be explained by the thickness of the skin but by the higher water content per surface area. Water in the skin of younger patients is predominantly bound to proteins supporting the structure and mechanical properties of the (glycol-)proteins[224].

Glycoproteins, such as hyaluronic acid, can bind up to 1000 times their volume in water due to their property of high negative charge resulting from the presence of carboxyl and sulphate groups.

In aged skin the water content of the skin not only increases but also changes form in which it is stored. Whereas in young skin a lot of water is bound to these glycoproteins and relative few water molecules are bound to each other in a tetrahedron form, the increased numbers of glycoproteins in older skin lack the same capability to bind water. In aged skin glycoproteins show altered protein conformation with increased folding and are deposited on the elastotic material of the superficial dermis, and not between collagen and elastic fibres as in normal skin. The change in structure and location makes the glycoprotein more hydrophobic resulting in relative high amount water bound to itself in the tetrahedron form[224]. The exact redistribution of water in aging skin remains unclear but many agree on the increase of total water content. Consistent with these findings, the total water content of the dermis is significantly higher in aged skin than young adult skin[122] despite the anticipated reduction due to the decreases in dermal thickness with age[225, 226].

#### 6.1.4. Arm TDC and BMI

Obese (BMI > 30 kg/m<sup>2</sup>) women are 3.6 times more likely to develop BCRL [33, 227]. Average BMI in our patient group was 29.7 kg/m<sup>2</sup>. We did not find any correlations between absolute (and the ratio of) TDC values and BMI. In a group with 68 healthy women Mayrovitz et al describes a trend of decreasing average TDC in the arm with increasing BMI [123]. At a measurement depth of 2.5mm and 5.0mm there was a significant difference in TDC values when healthy female participants with a normal BMI (< 25 kg/m<sup>2</sup>) were compared with overweight (25 – 29.9 kg/m<sup>2</sup>) and obese (> 30 kg/m<sup>2</sup>) women. Note that, in this research, the skin at the ventral side of the arm was investigated instead of the thicker skin at the dorsal aspect. This could potentially explain the lower TDC values at deeper measurement depths since a smaller proportion of the electromagnetic pulse is reflected by the high water content dermis and epidermis.

The development of adipose tissue involves remodelling of the extracellular matrix [228] and an increase in number and size of adipocytes [229]. Changes in composition of the extracellular matrix are highly associated with an increased risk of accumulation interstitial fluid [230]. Furthermore, total adipose tissue water is believed to increase with increasing adipose mass [206, 231]. These alterations in subcutaneous tissue in obese women increase the conductivity and would in theory lead to higher TDC readings at deeper measurements.

Mellor et al. [93] discovered in a small study using ultrasound to determine skin thickness that there's a positive relation between skin thickness and subcutis thickness in arm affected by BCRL, but negative relation in the control arm (*see appendix 3*). An increase of subcutaneous fat was related to a decrease in skin thickness in healthy tissue of the contralateral arm of BCRL patients. This also implies that the results of Mayrovitz et al. obtained in group of healthy women cannot be extrapolated to patients diagnosed with BCRL due to different skin to subcutis ratios. Before a linear correlation between BMI and TDC can be determined, especially in patients with BCRL, more research is desirable.

### **6.1.5. Arm TDC related to surgery type and self-reported measures**

Most studies use subjective self-reports as a way to determine lymphedema in breast cancer survivors. Often self-assessment questionnaires are designed to get information on pain, perception of swelling, heaviness, impingement and quality of life. Not only is self-assessment moderately reliable[34, 69], self-assessment is also limited in that it is unable to detect subclinical disease. In this study, we found significantly higher TDC ratios in patients who experienced pain and discomfort (see *table 4*) but no significant differences in arm volume ratio. A similar trend could be spotted for patients with a perceived swelling, although these results were not significant. We did not find any correlation between experienced heaviness of the arm and TDC or volume ratio. None of the self-reported questionnaire items could be related to the volume measurements. There is need for reservation when assessing lymphedema using subjective self-assessment questionnaires. In a study including 136 breast cancer patients treated with either SNB or ALND, only 41% of the patients reporting swelling or discomfort had measured increased volume[34]. This discrepancy raises the question of whether patients tend to overestimate their condition or that currently used volume assessment methods are not accurate enough to detect small changes in tissue composition. Unfortunately both hypotheses are equally bad for the patient's prognosis. People with clinically symmetrical arms reporting feelings of discomfort, heaviness and swelling are at risk of missing essential treatment in the early subclinical phase of their disease potentially averting severe complications. On the other hand, if there is clinically evidence to exclude lymphedema, patients with serious subjective discomfort should be referred for further investigations to detect the origin. With larger randomized controlled trials TWC assessment using the TDC could potentially fill that void in the diagnostic process.

When dividing our population by surgical treatment type, we found significantly higher TDC ratios in patients treated with a mastectomy. Women treated with a mastectomy have an increased risk of developing BCRL over women treated with breast conservative therapy[25, 29, 30].

Sentinel lymph node Biopsy (SNB) has replaced axillary lymph node dissection (ALND) as the standard method of axillary staging for women with (early-stage) breast cancer. The introduction of SNB diminished morbidity such as BCRL as proven by several groups[21, 31-36]. We didn't find any correlation between removal of lymph nodes and TDC. Unfortunately we must admit that due to small numbers and missing information our results regarding lymph node dissection are not reliable. A prospective study comparing number of nodes removed with TDC values over time is recommended.

## **6.2 Breast lymphedema**

According to existing literature, approximately one out of every three women treated for breast cancer suffer from a changed sensation in the operated breast [47, 48]. Often these signs are attributed to lymphedema without providing evidence for this hypothesis, mainly because there is no objective way of measuring lymphedema in the breast. The ability to detect small changes in TWC makes the MMD an adequate tool to objectively quantify lymphedema in the breast. To our knowledge this is the first study that provides evidence that TDC measurements can be practically used for the assessment of breast lymphedema (BLE) after breast cancer treatment. We found significantly different TDC values between operated and control breast in all four quadrants in both mastectomy and BCT group (see *table 2*).

### **6.2.1. Localization of measurement**

When comparing each quadrant of the affected breast individually with the corresponding quadrant in the control breast we found significantly higher TDC values, in spite of great variation in TDC values between quadrants.

In the control and post-BCT breast we found significantly higher TDC values in the lower two quadrants compared to the upper quadrants. In the post-mastectomy breast a similar trend is spotted, although these differences are not significant. Since all measurements were done with the

patient in an upright position we hypothesize that fluid accumulates in the lower quadrants of the breast due to gravitational influence. This might explain the higher TDC values in the lower parts of the breast especially when breast tissue was preserved. Patients in the mastectomy group are missing natural weight of the breast and the infra-mammary fold, therefore the anatomical structure including natural ptosis of the breast is lacking. This absence of a natural lowest point of the breast can explain the similarity of TDC values in the upper and lower parts of the post-mastectomy breast. Furthermore, the dermal thickness in the lower quadrants of the breast is significantly greater than the upper quadrants[46, 232] (*See appendix 4*). Therefore a larger proportion of the electromagnetic waves are reflected by the relative high water containing dermis and a smaller part by the low water content subcutis, resulting in higher TDC values.

When dividing the control, post-mastectomy or post-BCT breast with a sagittal line higher TDC values were measured in the medial part of the breast, with the highest readings in lower medial quadrant (LMQ). The limited amount of research reporting on dermal thickness of untreated breast tissue shows great discrepancy. Wratten et al.[233] measured significantly thicker skin at the medial side of both control and BCT breast. This is partially supported by Rönkä et al.[46] for the upper quadrants and is contradicted by the findings of Ulger et al. [232] who found a greater dermal thickness on the lateral side of the breast. The greater contribution of reflected waves by the high water content dermis should theoretically result in higher TDC readings on the side of the breast where the skin is the thickest.

In the unaffected breast the majority of lymph nodes are located in the (upper) lateral quadrant, explaining better drainage of lymphedema in that region compared to the medial side of the breast. Furthermore, all of the quadrants, including the LMQ, are highly dependent upon the axillary lymph nodes in the upper lateral part of the breast for the major part of their lymph drainage[234, 235]. In our study, only three out of eighteen patients were treated with ALND. Preservation of lymph nodes in the (upper) lateral quadrant in most patients may result in better drainage and less TWC.

Nevertheless, it is probable that the breast cancer surgery obliterated essential parts of the natural

anatomy of the lymphatic system in the breast, with the highest risk of compromising the drainage from the LMQ to the axillary lymph node chain. Using ultrasound, Rönkä et al.[46] reported more skin thickening in patients treated with a complete ALND compared to SNB. Despite measuring higher dermal thicknesses in the medial quadrants of breasts treated for breast cancer with BCT, control breasts showed highly thicker skin on the lateral side of the breast. A higher increase of medial skin thickness than at the lateral side after surgery was also reported by others[233]. Alterations in lymph drainage patterns and backflow of lymph fluid away from the axilla might be responsible for this post-surgery shift in skin thickness.

### **6.2.2. Breast skin TDC and probe depth**

The top layer of breast skin is constructed by a slightly smaller stratum corneum (~0.010mm) and a relative bigger percentage of high water content epidermis reaching to approximately the same depth as in the arm skin. Reports on thickness of the underlying dermis vary significantly, but most report equal or slightly greater (~1.50mm – 2.0mm) skin thickness in the breast (*see appendix 4*). Theoretically, all of the waves at a measurement depth of 1.5mm are completely reflected by the water rich (epi-)dermis, resulting in the highest absolute TDC values in the control breast. This trend is spotted in all quadrants with the exception of the UMQ, because the skin is relatively thinner in the upper quadrants. Therefore the part of the electromagnetic wave created by the 1.5mm reflected by the low water content subcutis is higher than the 0.5mm probe[232]. All TDC values in the control breast differed significantly depending on measurement depth, except when comparing the 0.5mm and 2.5mm probe. This can potentially be explained by the fact that 0.5mm probe in most quadrants does not reach into the basal cell layer of the deeper dermis where most skin water is found and that a large part of the 2.5mm probe penetrates in the relative dryer subcutis, both lowering the TDC. This is supported by the highest average TDC values found at a depth of 1.5mm in the lower medial quadrant where the skin is believed to be the thickest[232, 233, 236]. The TDC values measured with

the 5.0mm probe were all relatively lower, because the levels are negatively influenced by the subcutaneous fat which has very low water content of just 10%[192].

Surgery and subsequently lymphedema are known to increase the skin thickness from ~0.2mm up to ~2.5mm, depending on breast cancer treatment modality and assessment method[46, 233, 237-239].

In the post-BCT breasts, the highest TDC values were found at depths of 0.5mm and 2.5mm with no significant difference between them. The highest ratios between affected and control breast were found with the 2.5mm probe, with the exception of the ULQ. These findings are in line with the fact that the most significant changes in TWC take place in the deeper layer of the dermis and the top layer of the subcutis[204-206] and that the dermis-subcutis boundary increases to an absolute depth of ~1.9 – 3.6mm after breast cancer treatment (*see appendix 4*), especially in the lower quadrants of BCT breasts. Interestingly, the measurements taken with the 5.0mm probe differed from all other probes except the 1.5mm probe. This remarkable result is predominantly caused by the relative low TDC values of the 1.5mm probe on the lateral side of the breast.

In line with previous findings of breast cancer related lymphedema of the arm[25, 50], it is reasonable to assume that risk of developing breast lymphedema is significantly higher after mastectomy compared to BCT. This risk will increase even more by the higher number of lymph nodes removed in the mastectomy group. Surprisingly, the TDC values, particularly in the lower quadrants, were considerably lower than in the BCT group. Albeit lower than expected, all but one TDC values in the post-mastectomy breast were still significantly higher than the control, underlining the ability of the MMD to detect lymphedema. The distribution of TWC through the mastectomy skin was also more equal, probably because there is no longer a basin in the lower quadrants normally created by the gravity pulling down the breast tissue. This more widespread distribution of lymphedema might be responsible for the lower TDC values. Unfortunately, there is not much known about post-mastectomy skin thickness. We believe that more research on the thickness and water distribution in post-mastectomy skin is necessary before conclusions can be drawn regarding effective measurement depths.

### **6.2.3. Breast TDC correlated to age and BMI**

In contrast to the TDC assessments in the arm, no correlation was established between TDC values of the breast and age. The main difference between (dorsal) arm skin and breast skin is the amount of photo exposure with increasing age. Where photo exposed skin thins with age, some believe that there is little change or even an increase of dermal thickness in protected areas[215]. Contradicting this are the results presented by Ulger et al.[232], who found a significant decrease in dermal thickness with age in all quadrants. To our knowledge there is no information available on the development of proteins and redistribution of water molecules in the interstitial space of breast skin in relation to age. Furthermore, older patients have a higher risk of ptosis of the breast due to loss of mammary parenchyma in all probability affecting the water distribution between different areas in the breast and potentially influencing the thickness of the skin.

Obese (BMI>30 kg/m<sup>2</sup>) women are significantly more likely to develop BCRL in the arm[33, 227], but no numbers are available for women developing breast lymphedema (BLE). Although not supported by our research another group did find a significant correlation between BMI and TDC values in the arm[123]. Average BMI in our patient group was 29.4 kg/m<sup>2</sup>. We did not find any correlation between BLE and BMI. Hypothetically there is reason to believe that changes in the composition of extracellular matrix and adipose tissue water content will occur with increasing BMI but a prospective study with more patients is necessary before the correlation between BMI and TDC can be determined.

### **6.2.4. Breast TDC related to surgery type and self-reported measures**

Many studies use subjective self-reports as a way to determine the presence of lymphedema in breast cancer survivors. Often lymphedema self-assessment questionnaires are designed to get information on pain, perception of swelling, heaviness, impingement of the arm and quality of life, but there no well known questionnaires dedicated to assess breast morbidity. Not only is self-

assessment moderately reliable [34, 69], self-assessment is also limited in that it is unable to detect subclinical disease.

In our research we found that the majority of patients reporting pain were treated with BCT and had significant higher TDC ratios in the lower later quadrant at the deeper measurements. Interestingly enough, only two of the eleven patients reporting pain were operated in the lower lateral quadrant. The others had either a mastectomy, or surgery, in the upper part of the breast. Patients reporting discomfort also showed higher TDC ratios in this part of the breast, supported by a non-significant trend at the lower measurement depths. Additionally, higher TDC ratios were found in the ULQ at several depths. Of the patients suffering from discomfort eight of them also experienced a swelling of the affected breast. Perception of increased size was highly correlated with high TDC values in the lower quadrants of the breast and especially on the lateral side, because of the accumulation of tissue water in the lower compartments of the BCT breast. This greater TWC in the deeper lateral part of the breast may have resulted in a higher number of patients reporting pain or discomfort. There was no correlation between TDC ratio and feelings of heaviness.

To our knowledge, there are no other studies investigating the relation between subjectively experienced lymphedema and an objective method that can detect changes in interstitial fluid associated with lymphedema. In the limited research that is done on BLE, Rönkä et al.[46] showed that even clinical assessment of the affected breast by a professional underestimated the real incidence of lymphedema measured by ultrasound. Similarly to self assessment methods used to determine arm lymphedema, caution is needed when interpreting subjective BLE questionnaires.

### **6.3 Clinical cut-off point for arm and breast lymphedema**

A huge disadvantage of most methods commonly used to assess BCRL and BLE is that they require individual normalisation using a control arm or pre-surgery measurements. This limits their use to unilateral breast cancer and makes the procedure more time consuming. We believe that the TDC measurement method shows great potential to determine a clinical cut-off point in the future,

making control measurements superfluous. Nevertheless collection of much more reference data of healthy tissue is necessary before this is possible. Larger numbers need to be measured in order to make valid sub-group analysis so that the clinical cut-off point can be corrected for age, BMI and anatomical site.

#### **6.4 Future research**

We believe that the TDC method holds great potential and can be used in future research to quantify lymphedema even in a subclinical stage. As described above, more research needs to be done before we can determine a clinical TDC cut-off point which determines the presence of lymphedema without the need for comparison with the contralateral arm.

The next step in the investigation of the TDC method as a lymphedema assessment tool would be a randomized clinical trial in which the influence of the different breast cancer treatment modalities and the outcome of early treatment versus late treatment of lymphedema could be compared.

Preoperative baseline measurements at the day of surgery consisting of TDC and volume assessment combined with subjective self-reports using the questionnaire as found in the appendix 1, will be followed up by repetition of these measurements at 2, 6, 12 and 24 months. Intervals may differ slightly depending on different adjuvant breast cancer treatments and availability of the patient (see appendix 5). The aim is to collect information on skin thickness of each individual patient using ultrasound before the treatment begins, since dermal thickness influences the TDC readings.

Besides monitoring the possible onset and development lymphedema following the different aspects of breast cancer treatment, we will be able to select a group of people with early elevated levels of TDC at the baseline measurement or early in the follow up, even when these patients do not have subjective complaints yet. We will randomize this population into two different groups. One group will immediately receive lymphedema treatment using compression garments even when subjective feelings of lymphedema are absent and a second group in which the start of lymphedema treatment is postponed until the moment when the patient presents herself with subjective feelings associated

with lymphedema. The outcome will be defined as swelling of the arm, subjective sensations of lymphedema and raised TDC levels in the long term follow up. A third group of patients with normal TDC levels in early measurements will not be randomised and will only receive lymphedema treatment when swelling is present or when patients develop complaints associated with lymphedema. After 12 and 24 months we can determine whether early intervention in a population indentified by the TDC method as high risk is useful. Furthermore, we could observe the prognostic value of an early elevated TDC level by comparing group 2 and 3. See the flowchart in appendix 6.

## **6.5 Conclusion**

No standard guidelines for the diagnosis and assessment of BCRL exist. Although the true etiology of lymphedema remains unknown, radiation, chemotherapy, type of breast surgery, and extent of axillary surgery are commonly cited as risk factors. However, the numerical relationship between the type of treatment and the risk of lymphedema is not clearly correlated. This underlines the need for an objective quantitative method that can be used on a daily basis in the clinic for assessing lymphedema in an early stage. A sensitive objective method will not only help in identifying risk factors associated with lymphedema, but also improve treatment. Especially since women diagnosed with lymphedema in an early stage of the disease benefit more from the currently available lymphedema treatments and have better prospects[45].

This study demonstrates that measuring TDC using the MMD is an effective method for quantifying lymphedema in both arm and breast and is an important tool in detecting early TWC changes. However, this is a relatively small cohort trial. Although the trial was prospective in nature, TDC measurements were performed focused on a known diagnosis of lymphedema. Ideally, a prospective trial in which TDC measurements will be performed on a larger group of women treated for breast cancer.

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## List of publications

Assessment of lymphedema of the breast tissue following breast cancer related surgery using the tissue dielectric constant	R. Haen, C.E. Warnaby, J. Clarke, et al.	<i>Submitted</i>
Objective assessment and clinic cut-offs for breast cancer related arm lymphedema using tissue dielectric constant measurements demonstrate increased sensitivity to subjective report than traditional volumetric measures	C.E. Warnaby, R. Haen, J. Clarke, et al.	<i>Submitted</i>
The influence of variable skin thickness of the arm and breast in lymphedema on the tissues dielectric constant.	R. Haen	<i>Planned</i>

# Appendix

**Appendix 1**

**Lymphedema Data Collection**

Patient number:   
 Hospital number:

Patients initials	<input type="text"/>	Age	<input type="text"/>	Cup Size	<input type="text"/>			
Weight (Kg)	<input type="text"/>	Height	<input type="text"/>	BMI	<input type="text"/>			
Underbust	<input type="text"/>	Over bust	<input type="text"/>	Difference	<input type="text"/>			
Surgery	Right	<input type="checkbox"/>	Left	<input type="checkbox"/>	Bilateral	<input type="checkbox"/>		
	Mastectomy	<input type="checkbox"/>	Date of Surgery:					
	BCT	<input type="checkbox"/>	Quadrant:	ULQ	UMQ	LLQ	LMQ	
	Axillary sampling:	<input type="checkbox"/>	Axillary clearance:		<input type="checkbox"/>			
	Number of nodes:	<input type="text"/>	Weight of axillary specimen:		<input type="text"/>			
Date of assessment:	<input type="text"/>	Arm			Breast			
Pain rating:	0 1 2 3 4 5 6 7 8 9 10			0 1 2 3 4 5 6 7 8 9 10				
Discomfort: None/Mild/Mod/Severe	0 1 2 3			0 1 2 3				
Heaviness: None/Mild/Mod/Severe	0 1 2 3			0 1 2 3				
Perception of increased size: None/Mild/Mod/Severe	0 1 2 3			0 1 2 3				
Skin changes	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>	Yes	<input type="checkbox"/>	No	<input type="checkbox"/>

Date of initial clinic visit:	
Time between surgery and first visit:	
Time between first visit and today:	
Number of clinic visits to date:	

	Lymphedema treatment type	Length of treatments
<input type="checkbox"/>	Manual Lymphatic Drainage	
<input type="checkbox"/>	(Self-) Simple Lymphatic Drainage	
<input type="checkbox"/>	Compression Sleeve	
<input type="checkbox"/>	Bandaging	
<input type="checkbox"/>	Other:	

Satisfaction with the lymphedema clinic:	0 1 2 3 4 5 6 7 8 9 10
Success of treatment to date:	0 1 2 3 4 5 6 7 8 9 10

**Note.**

*Satisfaction:* Where 0 is entirely unsatisfied and 10 is the most satisfied. How satisfied are you with the care you have been given for lymphedema?

*Success of treatment:* Where 0 is 'The lymphedema has got significantly worse', 5 is 'Nothing has changed' and 10 is 'The lymphedema has disappeared'.

### Appendix 2

Author (year)	Assessment method	Subjects	Anatomical position	Stratum corneum (mm)**	Epidermis (mm)	Dermis (mm)	Total skin thickness (mm)
Crisan et al (2012) [194]	20MHz ultrasound up to 25 mm depth	160 patients  Man/Female (50/50)  a. <20 year b. 21-40 year c. 41-60 year d. 61-80 year	1. dorsal forearm 2. medial arm (3. zygomatic area)	<i>Not separately assessed.</i>  <i>Epidermis includes the stratum corneum in this study</i>	1a. 0.175 ±0.033 1b. 0.157 ±0.022 <b>1c. 0.178 ±0.038</b> <b>1d. 0.171 ±0.037</b>  2a. 0.155 ±0.030 2b. 0.162 ±0.121 2c. 0.157 ±0.040 2d. 0.177 ±0.043	1a. 1.209 ±0.272 1b. 1.279 ±0.294 <b>1c. 1.256 ±0.040</b> <b>1d. 1.220 ±0.223</b>  2a. 0.908 ±0.165 2b. 0.924 ±0.159 2c. 0.884 ±0.135 2d. 0.941 ±0.209	1a. 1.384 1b. 1.436 <b>1c. 1.434</b> <b>1d. 1.391</b>  2a. 1.063 2b. 1.086 2c. 1.041 2d. 1.118
Sandby-Møller et al. 2003 [195]	Histological assessment of 3mm punch biopsies + Cryo-preparation***	71 volunteers	1. dorsal forearm 2. shoulder (3. buttock)	1. <b>0.0183</b> (4.9 SD) 2. 0.0110 (2.2 SD) 3. 0.0149 (3.4SD)	1. <b>0.0566</b> (12.7) 2. 0.0703 (13.6) (3. 0.0815 (15.7) <i>Including s.corneum:</i> 1. <b>0.0749</b> (12.7) 2. 0.0813 (13.5) (3. 0.0965 (16.1))	<i>Not assessed</i>	<i>Cannot be calculated due to lack of report on dermis thickness.</i>
Werth et al. 1998 [196]	20MHz ultrasound	20 Healthy female Caucasian controls. (44-70 years old)	1. dorsal forearm 2. ventral forearm 3. upper arm	Only complete dermal thickness (mm) was measured: mean (+SD)  <b>1. 1.49 (0.05)</b> 2. 1.17 (0.02) 3. 1.02 (0.04)			<b>1. 1.49 (0.05)</b> 2. 1.17 (0.02) 3. 1.02 (0.04)
Koehler et al. 2010 [197]	multiphoton laser tomography, triple measurements)	30 volunteers  Average mean age ( ±SD) a. 23.3 ±1.9 b. 47.3 ±3.1 c. 72.1 ±6.4	1. dorsal forearm (10 cm proximal of wrist)	1a. 0.018 1b. 0.016 <b>1c. 0.025</b>	1a. 0.057 1b. 0.048 1c. 0.049 <i>Including s. Corneum:</i> 1a. 0.075 <b>1b. 0.064</b> <b>1c. 0.074</b>	Only <i>papillary dermis</i> was assessed:  1a. 0.103 <b>1b. 0.086</b> <b>1c. 0.095</b>	<i>Cannot be calculated due to lack of part of dermis thickness.</i>

Author (year)	Assessment method	Subjects	Anatomical position	Stratum corneum (mm)**	Epidermis (mm)	Dermis (mm)	Total skin thickness (mm)
Seidenari (1995) [198]	20MHz ultrasound	91 women  Age (in years): a. 27-31 (n31) b. 40-50 (n12) c. 60-70 (n18) d. 70-80 (n15) e. 80-90 (n15)	1. dorsal forearm 2. ventral forearm	Only complete dermal thickness (mm) was measured: mean (+SD)  1a. 1.29 ±0.18 1b. 1.23 ±0.18 <b>1c. 1.16 ±0.19</b> 1d. 1.11 ±0.25 1e. 1.06 ±0.26	2a. 1.02 ±0.18 2b. 1.06 ±0.14 2c. 1.07 ±0.17 2d. 0.94 ±0.15 2e. 1.11 ±0.26		1a. 1.29 ±0.18 1b. 1.23 ±0.18 <b>1c. 1.16 ±0.19</b> 1d. 1.11 ±0.25 1e. 1.06 ±0.26
Egawa et al. (2007) [199]	Confocal Raman spectrometry	33 Japanese volunteers. (6 male / 27 female)  Average age: Male: 43.2 y. Female: 56.3 y.	1. ventral side upper arm 2. ventral forearm	1. 0.0218 2. 0.0226  Ventral forearm female only:  2. 0.0184	<i>Not assessed</i>	<i>Not assessed</i>	<i>Cannot be calculated due to lack of report on epidermis and dermis thickness.</i>
Sauermann et al. (2002) [200]	Confocal Raman spectrometry (laser scanning microscope)	Healthy volunteers (skin type I-III)**** 2 groups, each 11 females / 2 males  Average age: a. 23.1 (18-25y) b. 72.5 (Range unknown)	1. ventral side forearm (middle)	1a. 0.010 ±0.0032 1b. 0.011 ±0.019	1a. 0.035 ±0.004* 2b. 0.040 ±0.005*  Minimal epidermis thickness measured	<i>Not assessed</i>	<i>Cannot be calculated due to lack of report on dermis thickness.</i>

Author (year)	Assessment method	Subjects	Anatomical position	Stratum corneum (mm)**	Epidermis (mm)	Dermis (mm)	Total skin thickness (mm)
Batisse et al. (2002) [201]	Confocal microscopy and 25MHz ultrasound	36 healthy women  Average age: a. 21 ( $\pm 2$ years, n18) b. 65 ( $\pm 2$ years, n18)	1. dorsal side upper arm	1a. 0.015 $\pm$ 0.003 <b>1b. 0.017 <math>\pm</math> 0.003</b>	1a. 0.077 $\pm$ 0.007 <b>1b. 0.069 <math>\pm</math> 0.010*</b>  <i>Including s. Corneum:</i> 1a. 0.092 <b>1b. 0.086</b>	1a. 1.2 $\pm$ 0.1 <b>1b. 1.4 <math>\pm</math> 0.4</b>	1a. 1.29 <b>1b. 1.48</b>
Huang et al. (2007) [202]	20MHz ultrasound (B-scan, triple measurements)	38 patients  a. who received therapeutic irradiation because of nasopharyngeal carcinoma Avg. Age: 54 $\pm$ 11  b. 20 controls, avg. Age 27 $\pm$ 3y	1. ventral side distal forearm (3cm from radiocarpal joint)*****	Only complete dermal thickness (mm) was measured: mean (+SD)  1a. 1.52 $\pm$ 0.24 1b. <i>No absolute dermal thickness reported. No significant difference with 1a. (p&gt;0.05)</i>			1a. 1.52
Moore et al. (2003) [193]	22MHz ultrasound (B-mode)	39 patients with systemic sclerosis) 34 healthy controls (5 male/29 female) Age 46 (26-74y)  Healthy controls: L. left R. right	17 sites on the body:  1. ventral forearm (10cm from ulnar styloid)  2. ventral upper arm (8cm from medial epicondyle)	<i>Stratum corneum not separately assessed.</i>	1L. 0.244 ( $\pm$ 0.061) 1R. 0.247( $\pm$ 0.056)  2L. 0.266 ( $\pm$ 0.062) 2R. 0.266( $\pm$ 0.062)	1L. 0.850 ( $\pm$ 0.111) 1R. 0.826 ( $\pm$ 0.144)  2L. 0.818 ( $\pm$ 0.109) 2R. 0.800 ( $\pm$ 0.130)	1L. 1.094 1R. 1.073  2L. 1.084 2R. 1.066

Author (year)	Assessment method	Subjects	Anatomical position	Stratum corneum (mm)**	Epidermis (mm)	Dermis (mm)	Total skin thickness (mm)
Ihn et al. (1995) [203]	30MHz ultrasound (B-mode) Quadruple measurements	(97 with systemic sclerosis)  81 healthy controls (9 male/72 female)  Age: 56.3 (22-76y)	1. dorsal side forearm	Only complete dermal thickness (mm) was measured: mean ( $\pm$ SD)  1. Control group (n=81) <b>0.91 <math>\pm</math>0.12</b> 1. SSc group (n=97) 1.24 $\pm$ 0.32			<b>0.91</b>

**Appendix 2: Overview of thickness of the components of normal arm skin.** The thicknesses printed in bold correspond the most with our study population in terms of gender, age and anatomical site. Results are presented per anatomical site (numbered in the 4<sup>th</sup> column) and different age group (indicated with a letter in the third column).

\* Significant difference (p<0.05)

\*\* Is in some studies part of the epidermis. Not all studies separate these two layers.

\*\*\*a special non-formalin processing technique in which the tissue is instantly frozen with isopentane (2-methylbutan), in order to prevent crystallization in the water content, and to minimize changes in the skin structure and epidermal thickness during the different preparation steps.

\*\*\*\* Skin color: Type I: Very light or white, Type II: Light or light-skinned European, Type III: Light intermediate or Southern European and East Asian, Type IV: Dark intermediate, also sometimes called "Mediterranean", Type V: Dark skin

\*\*\*\*\*Non-radiated control side in this study for the patients with nasopharyngeal carcinoma.

### Appendix 3

Author (year)	Assessment method	Subjects	Anatomical position	Complete dermal thickness (mm):mean (+SD)	Subcutis (mm)
Tassenoy et al. [95]	10 MHz ultrasound + MRI (T2, with fat-subtraction)	7 Breast cancer patients with BCRL.** Average age: 60 (41-74) years.  85% mastectomy 100% ALND** Swelling: 38.4 months  a. affected arm c. control arm	1. ventral** * side upperarm 2. ventral side prox. forearm 3. ventral side distal forearm 4. dorsal side upperarm 5. medial side prox. forearm 6. medial side distal forearm	1a. 1.97 (0.41)      1c. 1.51 (0.21)* 2a. 1.93 (0.40)      2c. 1.36 (0.15)* 3a. 2.24 (0.37)      3c. 1.46 (0.12)* <b>4a. 2.10 (0.29)</b> <b>4c. 1.70 (0.24)*</b> <b>5a. 2.09 (0.25)</b> <b>5c. 1.66 (0.17)*</b> <b>6a. 2.19 (0.25)</b> <b>6c. 1.63 (0.41)*</b>  *Skin thickness on all sites is significantly (p<0.05) higher in the affected arm.	1a. 17.2 (2.39)      1c. 13.34 (4.20)* 2a. 18.44 (4.73)      2c. 12.77 (6.03)* 3a. 17.21 (5.16)      3c. 11.65 (5.43)* <b>4a. 25.34 (10.48)</b> <b>4c. 18.13 (10.54)*</b> <b>5a. 15.83 (3.57)</b> <b>5c. 9.03 (5.44)*</b> <b>6a. 12.50 (7.84)</b> <b>6c. 7.84 (4.08)*</b>  *Skin thickness on all sites is significantly (p<0.05) higher in the affected arm.
Mellor et al. [93]	20 MHz ultrasound for the skin (13.42 wide + 22.40mm deep) and 7 MHz ultrasound for the subcutis (4cm wide and 4cm deep).	10 Breast cancer patients with BCRL.** Average age: 59 (48-75) years.  30% mastectomy 70% BCT** 50% ALND  a. affected arm c. control arm	1. Ventral/ anterior side forearm 2. Lateral side forearm 3. dorsal side forearm 4. Medial side forearm 5. Average.	1a. 1.83 (0.13)      1c. 0.93 (0.13)* 2a. 1.87 (0.93)      2c. 1.12 (0.16)* <b>3a. 2.11 (0.65)</b> <b>3c. 1.48 (0.45)*</b> 4a. 2.08 (1.26)      4c. 0.96 (0.13)* 5a. 1.97 (1.00)      5c. 1.12 (0.14)*  *Skin thickness on all sites is significantly (p<0.0001) higher in the affected arm.	1a. 9.68 (5.34)      1c. 7.44 (3.06)* 2a. 7.44 (5.81)      2c. 4.73 (2.13)* <b>3a. 11.18 (7.28)</b> <b>3c. 2.77 (1.75)*</b> 4a. 12.96 (5.55)      4c. 7.39 (3.58)* 5a. 10.32 (5.64)      5c. 5.58 (2.04)*  The degree of increase in subcutis thickness varied significantly between the different sites (p < 0.0001). The dorsal aspect showed a greater increase the other aspects ( p < 0.01)

Author (year)	Assessment method	Subjects	Anatomical position	Complete dermal thickness (mm):mean (+SD)	Subcutis (mm)
Van der Veen et al. [240]	Ultrasound (frequency unknown)	22 Breast cancer patients with BCRL. ** + 9 healthy controls. (age unknown)  a. affected arm c. control arm	1. upperarm 2. Forearm ****	1a. 4.1 (0.4)                      1c. 3.6 (0.40)* 2a. 3.5 (0.8)                      2c. 3.1 (0.60)*  <i>These findings are extremely high without an explanation.</i>	1a. 14.0 (0.5)                      1c. 9.9 (0.47)* 2a. 7.3 (0.32)                      2c. 4.8 (0.23)*
Williams et al. 2002 [92]	20MHz ultrasound	59 Breast cancer patients with BCRL. ** Age: 59 ±2 years  a. affected arm	1. Ventral side forearm (8cm below elbow) 2.lateral shoulder	<b>1a. 2.37</b> 2a. 2.47  <i>No control arm assessed</i>	<i>Not assessed</i>

**Appendix 3. Overview differences in skin thickness between lymphedema affected arms and control.** The thicknesses printed in bold correspond the most with our study population in terms of gender, age and anatomical site. Results are presented per anatomical site (numbered in the 4<sup>th</sup> column) and different age group (indicated with a letter in the third column).

\* Statistical significant difference.

\*\*BCRL = Breast cancer related lymphedema. ALND = Axillary lymph node dissection. BCT = Breast conserving therapy.

\*\*\*Measurement points: 1, 10 cm above the cubital fossa on the anterior side; 2, 5 cm below the cubital fossa on the anterior side; 3, 10 cm below the cubital fossa on the anterior side; 4, 10 cm above the olecranon; 5, 5 cm below the olecranon; 6, 10 cm below the olecranon; 7, on the dorsum of the hand.

\*\*\*\*Images were taken from both the upper and lower arm, at 10 cm proximal and distal from the olecranon. Aspect of the arm is unknown.

#### Appendix 4

Author (year)	Assessment method	Subjects	Anatomical position	Stratum corneum (mm)	Epidermis (mm)	Dermis (mm)
Monteiro-Riviere et al. 1997 [241]	Histological assessments of 1mm skin samples	Unknown (skin obtained via skin tissue bank)	Breast. Details unknown	0.006 – 0.0011	0.045 – 0.065	2.000
Norlén et al. 1997 [242]	Histological assessment of skin specimens prepared with phosphate-buffered saline with the epidermal side down for 2 hours.	20 healthy females undergoing reconstructive surgery	Breast. Details unknown	Dry: 0.014 ±0.0035  After incubating in water (90min): 0.017 ±0.036  (26,3% ±16.3% increase)	<i>Not assessed</i>	<i>Not assessed</i>
Menon et al. 2007 [243]	Histological assessment of 4mm punch biopsies	20 healthy females undergoing reconstructive surgery	Breast. Details unknown	0.010 – 0.015	0.050 – 0.150	<i>Not assessed</i>
Riecki et al. 1999 [237]	Histological assessment of 4mm punch biopsies + fixed in 10% formalin, embedded in Paraffin (three samples each site)	20 Breast cancer patients treated with radiotherapy Age: 55 (42-75y) Time since RTx: 26 (10-96 months)  a. affected c. control	Breast (side and quadrant not further specified)  1. at least 2cm above scar 2. scar tissue 3. at least 2cm below scar.	Only complete dermal thickness (mm) was measured: mean (±SD)  <b>1a. 1.88 ±0.35</b> <b>2a. 1.87 ±0.31</b> <b>1c. 1.63 ±0.21*</b> <b>3a. 1.84 ±0.27</b>  <i>1c. Measurement taken from equivalent area of 2 cm above the scar in the not treated control breast</i>		

Author (year)	Assessment method	Subjects	Anatomical position	Complete dermal thickness (mm)	Comments
Warzawski et al 1997 [238]	20MHz ultrasound (A-scan)	29 breast cancer patients  23 BCT** 6 mastectomies  Age: 58 (39-72y)	Breast mid-clavicular line 2-3 above (former) mamilla.	Only complete dermal thickness (mm) was measured: mean ( $\pm$ SD)  Affected early assessment (<3 months) 2.683 $\pm$ 0.721 Affected late assessment (median 30 months) <b>2.307 <math>\pm</math>0.934</b> Non-irradiated control breast skin. <b>1.683 <math>\pm</math>0.308</b>	
Wong et al. 2011 [236]	14MHz ultrasound (B-scan)	32 Asian breast cancer patients treated with radiotherapy (between 46 – 50 Gy)  Age: 53 (37-68y)	Breast at 9 points.	Only complete dermal thickness (mm) was measured: mean ( $\pm$ SD) of all nine sites.  Affected side: 0.17 $\pm$ 0.03      Control side: 0.18 $\pm$ 0.04  <i>Medial side was significantly thicker than lateral side in the affected and control breast. No absolute figures are reported: 0.19mm(medial) vs 0.17mm (lateral)</i>	NB! We believe 0.17 and 0.18 are probably not reported correctly. Probably the author meant 1.7mm and 1.8mm.
Liu et al. 2009 [239]	12MHz ultrasound (B-mode)	18 Breast cancer patients treated with radiotherapy (between 60 – 66.4y)  Time since RTx: median 22 (6-92 months) Age 56 (44-74)  a. affected c. control	Breast 4 points (12h, 3h, 6h, 9h)  1. average of all 4 measurements	Only complete dermal thickness (mm) was measured: mean ( $\pm$ SD) of all four sites  <b>1a. 2.61 <math>\pm</math>0.52</b> (range 1.53 to 3.65) <b>1c. 2.05 <math>\pm</math>0.22 *</b> (range 1.66 to 2.41)	<i>Although 4 sites in each breast were measured, the separate results are not reported.</i>



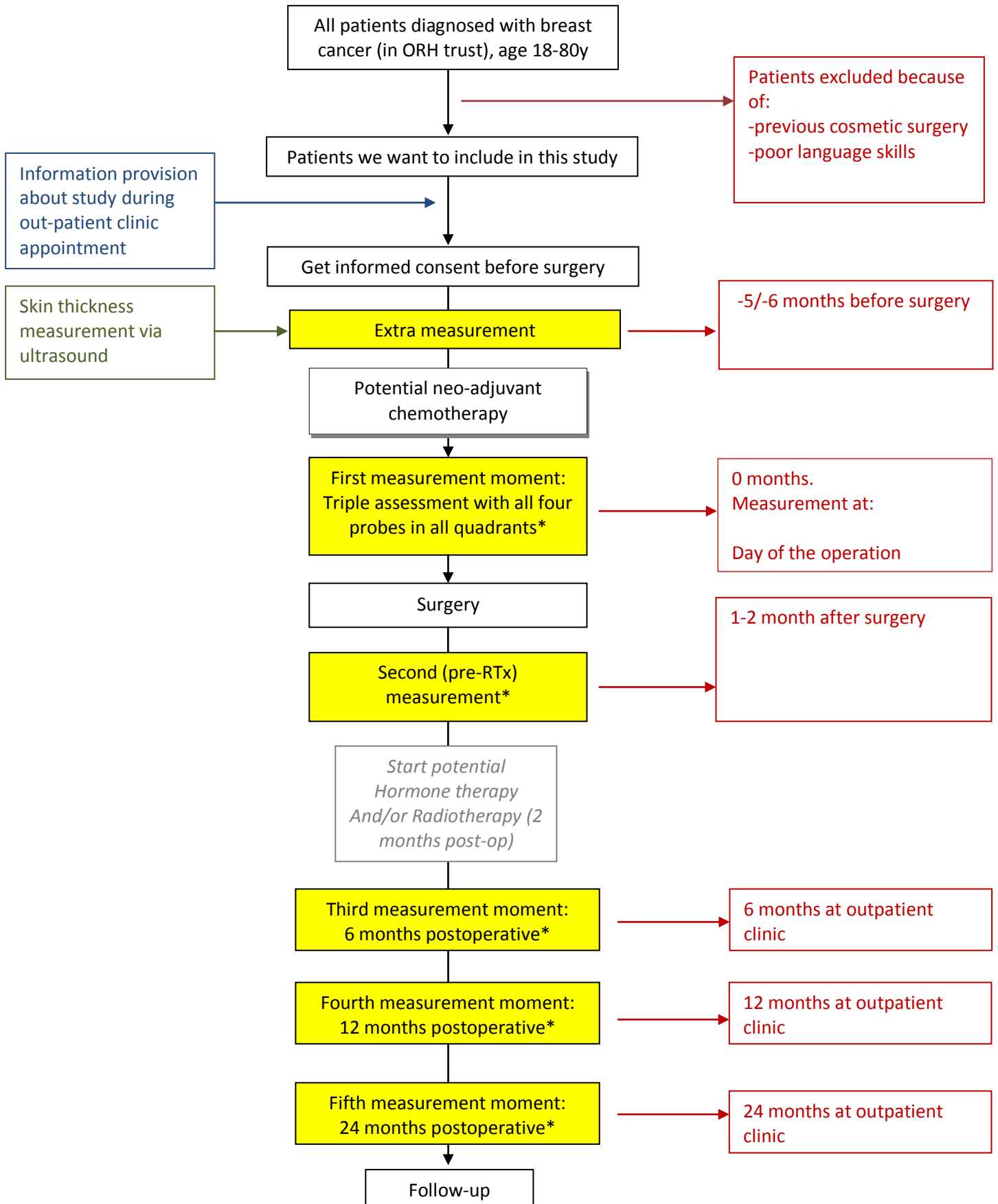
Author (year)	Assessment method	Subjects	Anatomical position	Complete dermal thickness				Comments
Rönka et al. 2004 [46]	5 – 13 MHz ultrasound	160 Scandinavian patients breast cancer patients treated with BCT (wide excision) + radiotherapy (n=157, 50 -52Gy)  Time since BCT: 12.6 (11.3-18.8 months)  SNB 59 (39-77 years)n=36  ALND+ 58 (37- 80 years) n=29  ALND- 58 (39 -81 years) n=37  a. affected c. controls	Breasts in 4 quadrants  1. ULQ 2. UMQ 3. LLQ 4. LMQ**	Only complete dermal thickness (mm) was measured: median (range)				48% of all patients suffered from clinically assessed lymphedema in the breast in the ALND+ group and 23% of the patients in the SND group.  Clinical signs, such as lymphedema, pigmentation, tenderness, were observed in the operated breast in 78% (124/160)
				<b>1a-SNB.</b>	<b>1.5 (1.0-4.4)</b>	<b>1c-SNB.</b>	<b>1.1 (0.7-2.5)</b>	
				<b>1a-ALND+.</b>	<b>1.8 (1.1-6.6)</b>	<b>1c-ALND+.</b>	<b>1.0 (0.6-1.6)</b>	
				<b>1a-ALND-.</b>	<b>1.9 (0.7-5.5)</b>	<b>1c-ALND-.</b>	<b>1.1 (0.6-1.1)</b>	
				<b>2a-SNB</b>	<b>1.7 (0.9-4.6)</b>	<b>2c-SNB</b>	<b>1.2 (0.9-2.9)</b>	
				<b>2a-ALND+</b>	<b>2.7 (0.9-6.1)</b>	<b>2c-ALND+</b>	<b>1.3 (0.9-1.7)</b>	
				<b>2a-ALND-</b>	<b>2.5 (1.2-6.5)</b>	<b>2c-ALND-</b>	<b>1.2 (0.7-2.1)</b>	
				<b>3a-SNB</b>	<b>1.9 (0.9-5.5)</b>	<b>3c-SNB</b>	<b>1.3 (0.7-2.1)</b>	
				<b>3a-ALND+</b>	<b>3.4 (1.1-7.5)</b>	<b>3c-ALND+</b>	<b>1.3 (0.8-1.9)</b>	
				<b>3b-ALND-</b>	<b>2.9 (1.4-6.4)</b>	<b>3c-ALND-</b>	<b>1.3 (0.8-1.9)</b>	
				<b>4a-SNB</b>	<b>2.1 (1.1-4.6)</b>	<b>4c-SNB</b>	<b>1.1 (0.7-2.2)</b>	
				<b>4a-ALND+</b>	<b>3.6 (1.1-7.5)</b>	<b>4c-ALND+</b>	<b>1.1 (0.7-1.6)</b>	
				<b>4a-ALND-</b>	<b>3.1 (1.4-6.4)</b>	<b>4c-ALND-</b>	<b>1.1 (0.7-1.7)</b>	
				*Skin affected breast per quadrant significantly thicker than corresponding site in control breast for all treatments.				
				Besides thicker skin in ALND group, significant more edema was assessed in the ALND group (p=0.029).				

**Appendix 4. Literature overview of thickness of the components breast skin.** The thicknesses printed in bold are corresponding the most with our study population in terms of gender, age and anatomical site. Results for the affected and control breast are presented per anatomical site (numbered in the 4<sup>th</sup> column). UMQ = Upper medial quadrant, LMQ = Lower medial quadrant, LLQ = Lower lateral quadrant and ULQ = upper lateral quadrant. SNB = Sentinel lymph node biopsy, ALND = Axillary lymph node dissection.

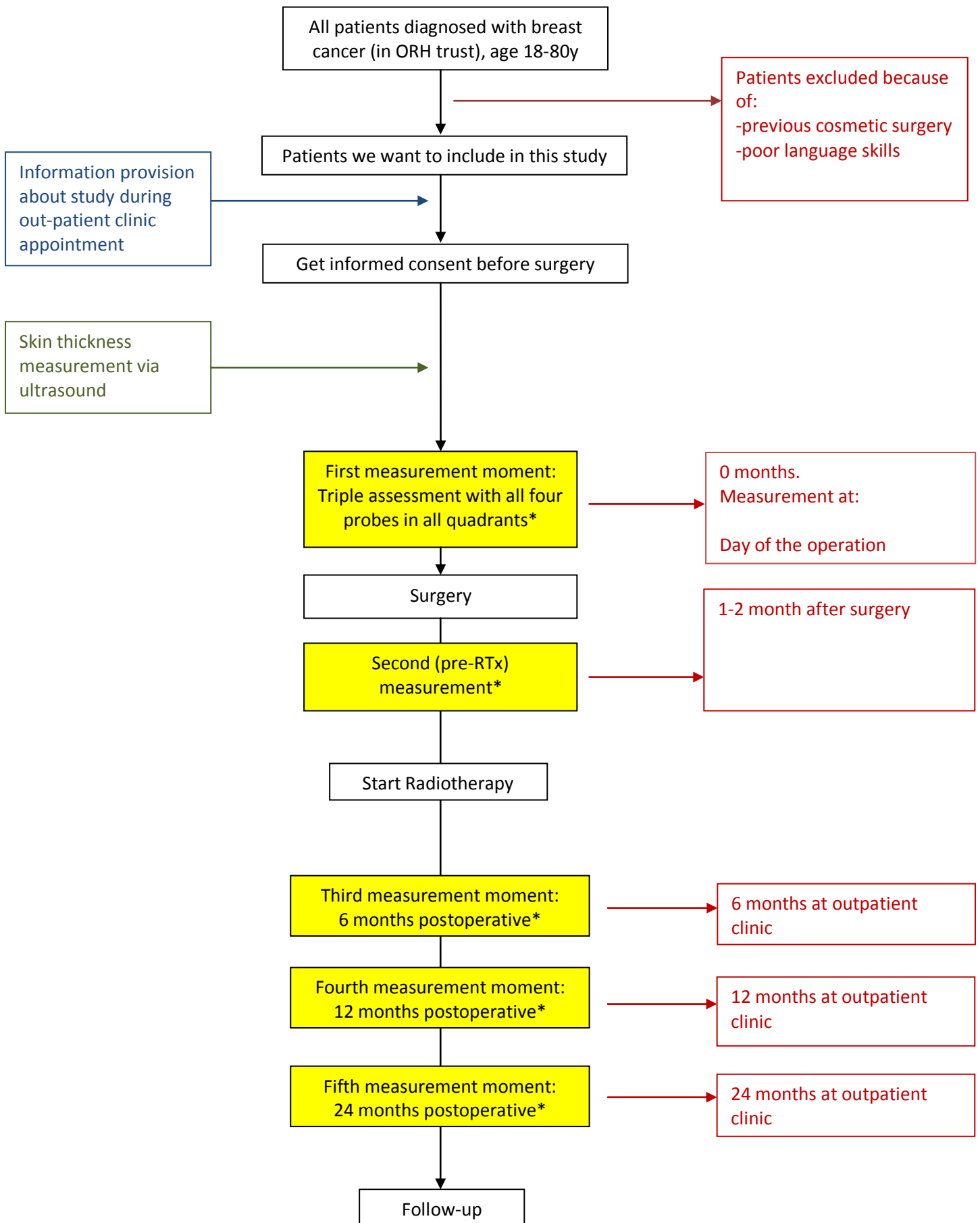
\* Significant difference (p<0.05)

## Appendix 5

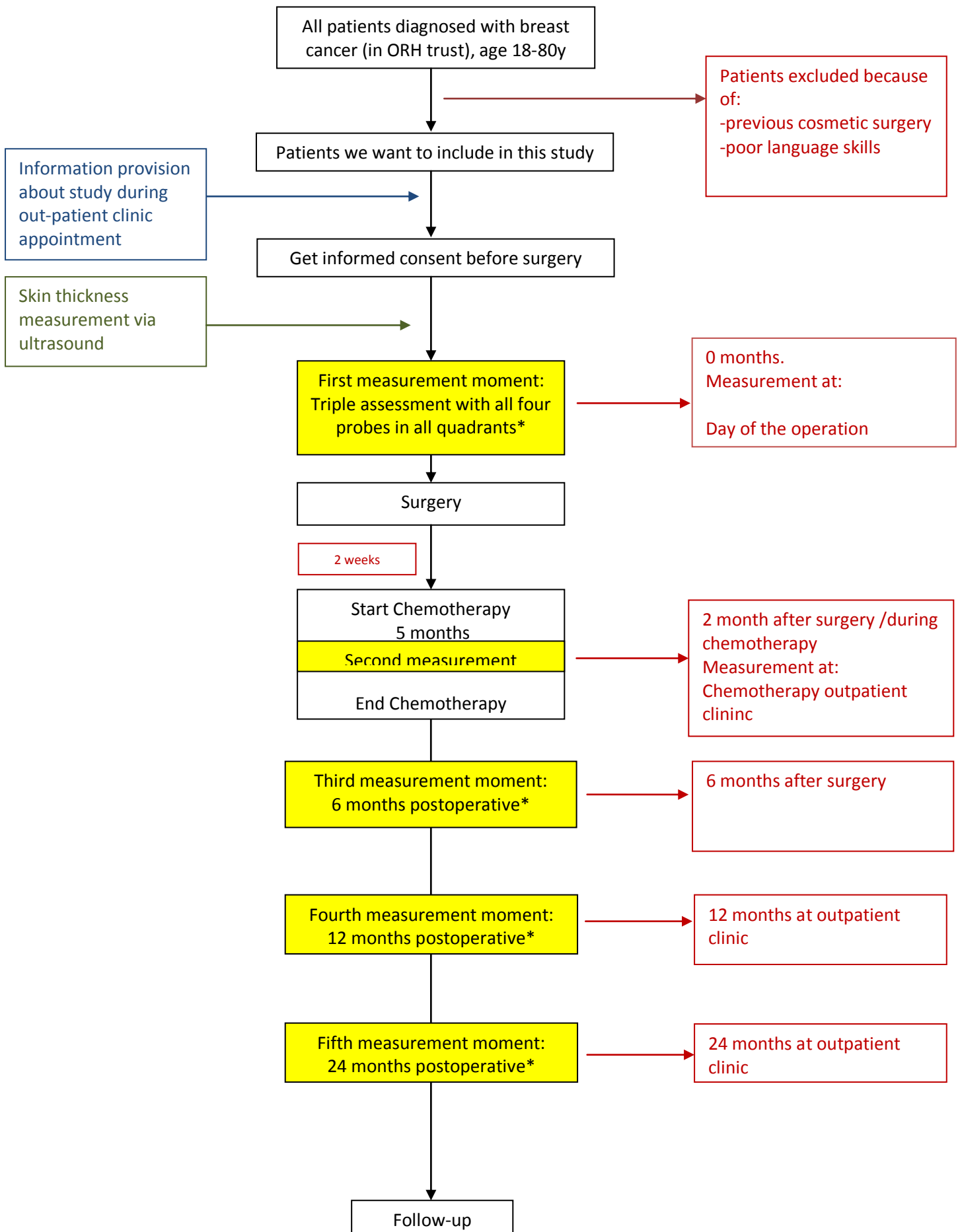
### GROUP 1 - Neo-adjuvant Chemotherapy



**GROUP 2 - Radiotherapy**

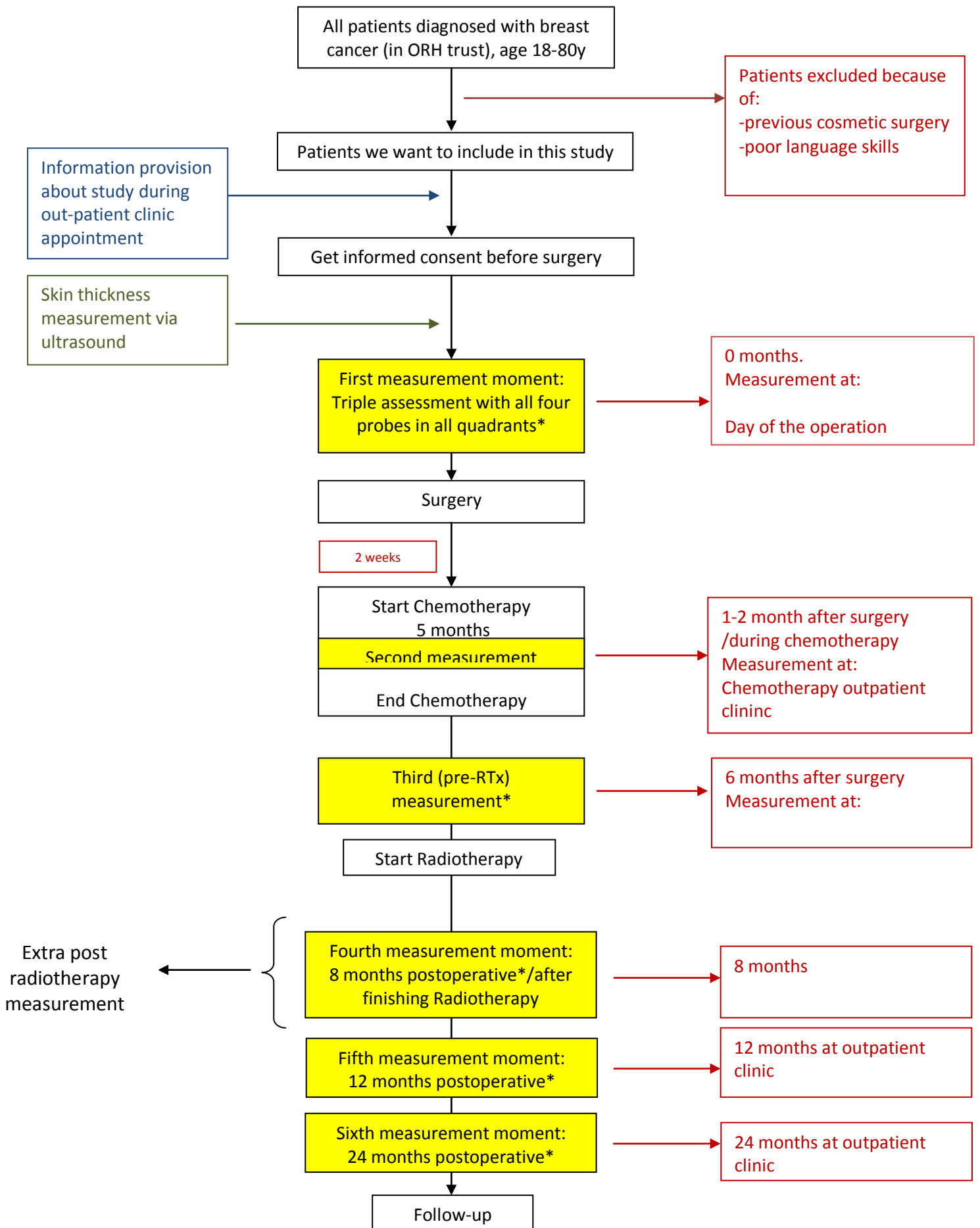


**GROUP 3 - Chemotherapy**



*\*Patients will be asked to complete the questionnaires before each measurement*

**GROUP 4 - Radiotherapy + Chemotherapy**



\*Patients will be asked to complete the questionnaires before each measurement

## Appendix 6

