Detecting the unseen:
Covert tissue changes in lymphatic filariasis and implications for the future of morbidity management.

Thesis submitted by

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5	Douglass, J., Graves, P., Lindsay, D., Becker, L., Roineau, M., Masson, J., Aye, N., Win, S., Wai, T., Win, Y., *Lymphatic Filariasis Increases Tissue Compressibility and Extracellular Fluid in Lower Limbs of Asymptomatic Young People in Central Myanmar*. Tropical Medicine and Infectious Disease, 2017. 2(4): p. 50.	J.D., S.G. and P.G. conceived and designed the study. L.B. and M.R. performed the laboratory analysis. N.N.A., S.S.W., Y.Y.W. and T.W. provided in-country advice and assisted with data collection. J.D., D.L. and J.M. analysed the data; J.D. wrote the manuscript with editorial input from all co-authors.

Statement of authorship

I confirm the above statement to my contributions to authorship are true and I consent to the inclusion of the relevant paper(s) in this thesis.

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Abstract

Introduction: Lymphoedema is a disfiguring and debilitating disease that cannot be cured. It occurs when lymphatic clearance from the tissue spaces is inadequate and is characterised by enlargement of the skin and subcutaneous tissue compartment. These pathological connective tissue changes can lead to gross deformity of limbs, significant social stigma, and reduced quality-of-life.

The majority of the global burden of lymphoedema is caused by the parasitic infection lymphatic filariasis (LF) and it is estimated that 17 million people are living with filariasis-related lymphoedema (FRL). The global program to eliminate LF (GPELF) aims to: interrupt transmission of the parasite using preventive chemotherapy (PC) delivered via annual mass drug administration (MDA) campaigns, and provide existing cases with adequate education and support in FRL management. Recommendations for FRL management in the GPELF have few strategies to reverse or halt early disease and no recognition of a latent or covert stage of disease. In contrast, treatment for breast cancer-related lymphoedema (BCRL) has moved from managing later stage disease with specially made compression garments, to preventive intervention as soon as a covert oedema is detected.

Covert lymphatic dysfunction has been detected in asymptomatic people with LF, but this has been achieved using expensive and invasive imaging methods. There is limited evidence that PC may reverse these covert changes but this is usually in research cohorts where everyone receives the medication, and not in real-life situations where community level coverage of PC during MDA is rarely 100%. Tissue tonometry is frequently used to measure changes in skin and connective tissue composition and bio-impedance spectroscopy (BIS) has been shown to detect latent BCRL. These devices are inexpensive, field-friendly, and well validated in BCRL but have had negligible use in FRL.

Objectives of this thesis: To determine if covert connective tissue changes can be detected in the lower limbs of young people living in a LF endemic region in central Myanmar, and the effect of annual PC medication on any changes found at baseline. Secondary outcomes were to: establish the reliability of the devices in the lower limbs of young people in Myanmar and Australia and identify moderating factors associated with variance in device scores.

Methods: Three tissue tonometers and one BIS device were used to measure tissue compressibility and free fluid in the lower limbs of young people in an LF endemic region in Myanmar. Data were collected before and after the annual MDA of PC medication and again after further PC had been offered to infected cases not covered by the MDA.

Results: All the devices were found to have good to excellent intra-rater reliability in the young Australian and Myanmar cohorts. Significant variations in device scores were associated with age, gender, BMI, hydration, and the female menstrual cycle. At baseline, LF-positive cases in Myanmar had clinically relevant and statistically significant increases in tissue compressibility at the non-dominant calf using the Indurometer, and in free fluid in the non-dominant leg using BIS. After the annual MDA, and with further PC offered to positive cases missed by the MDA, these between-infection group differences were not evident at follow-up, but the effect of time made this result unclear and further statistical modelling is required to clarify this.

Conclusions and implications: The Indurometer was able to detect a clinically relevant and statistically significant increase in tissue compressibility at the non-dominant calf in LF-positive, asymptomatic cases in Myanmar compared to their LF-negative peers. This single large significant result is supported by trends within and between other devices. These trends suggest the Indurometer and BIS as potential measures of connective tissue change in overt FRL cases and further study in this population is warranted. Evidence for the existence

of covert pathology associated with LF is increasing and these findings add another piece to that puzzle. This combined evidence supports the recognition of a stage 0 in FRL. Further recommendations for GPELF activities based on this thesis are: increased efforts to detect covert and early lymphoedema, and the instigation of enhanced morbidity management for early stage FRL.

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List of Abbreviations

ADLA	Adeno-dermato Lymphangitis
ANOVA	Analysis of Variance
BCRL	Breast Cancer-related Lymphoedema
BIS	Bio-impedance Spectroscopy
BMI	Body Mass Index
CDC	Centre for Disease Control
CDT	Combined Decongestive Therapy
cm	Centimetres
COV	Co-efficient of Variation
CRL	Cancer-related Lymphoedema
DDT	Dichloro-diphenyl-trichloroethane
DEC	Diethylcarbamazine Citrate
DMR	Department of Medical Research
ECF	Extracellular Fluid
EDTA	Ethylene-diaminete-traacetic Acid
ELISA	Enzyme-linked Immunosorbent Assay
Feb-15	February 2015
FRL	Filariasis-related Lymphoedema
g	Gram
GPELF	Global Program to Eliminate Lymphatic Filariasis
HREC	Human Research Ethics Committee

ICC	Intra-class correlation
ISL	International Society of Lymphology
JCU	James Cook University
Jun-15	June 2015
kg	Kilograms
KHz	Kilo-hertz
LED	Light Emitting Diode
LF	Lymphatic Filariasis
m	Metre
MDA	Mass Drug Administration
ml	Millilitre
mm	Millimetre
MMDP	Morbidity Management and Disability Prevention
MOH	Ministry of Health
MOHS	Ministry of Health and Sports
MOU	Memorandum of Understanding
N	Newtons
NTD	Neglected Tropical Disease
Oct-14	October 2014
Og4C3	Monoclonal antibody-based ELISA test for LF-antigen
PC	Preventive Chemotherapy
PHL	Public Health Laboratory

PNG	Papua New Guinea
PRISMA	Preferred Reporting Items for Systematic Reviews and meta-analysis
Ri:Re	Resistance Intracellular Fluid : Resistance Extracellular Fluid
RLV	Relative Limb Volume
rpm	Revolutions per minute
RPRG	Regional Program Review Group
SD	Standard Deviation
SE	Standard Error
ul	Microlitre
UK	United Kingdom
US	United States
USD	Unites States Dollars
VBDC	Vector Borne Disease Control
vs	versus
WHO	World Health Organization

Notes on language used in the thesis

At the time of commencing the study in Myanmar, the Ministry of Health was named Ministry of Health (MOH), this was changed during 2016 to Ministry of Health and Sports (MOHS) and both titles will appear in this thesis.

The term 'elephantiasis' is not a medical term. It is an eponym that is frequently used to describe advanced stages of lymphoedema where the limb is grossly enlarged, the skin has thickened and formed into deep folds, and there may be a dark, scaly layer – altogether a rather 'elephantine' appearance. Whilst I can appreciate the origin of the word, and have used it in the literature review in a historical context, I believe the term to be demeaning and stigmatising, and over the course of undertaking my doctoral studies have eliminated it from my own vocabulary. I am also concerned about its use in many publications where it is used in conjunction with lymphoedema, as in '… causes lymphoedema and elephantiasis…' as if they are two separate things. They are not.

I am also concerned about the conflation of the terms lymphatic filariasis (LF) and lymphoedema as if they are the same thing. This appears both in published literature and in social media where well-meaning tweets such as 'elephantiasis has been eliminated in ….' frequently appear. It is LF that is being eliminated, not lymphoedema. My concern is more than semantic, I am worried that lay persons – donors to be specific – are being given the impression that once LF transmission has be interrupted there will be no more to do. But lymphoedema is for life, and people with chronic disease will require our support for decades to come.

For these reasons you will not find the word elephantiasis in this thesis (after the refences in the literature review) nor the conflation of LF and lymphoedema as a single condition.

Chapter 1: Introduction to the thesis

Chapter 1 develops the questions, aims, and objectives that drove the research activities.

Chapter 2 reviews the relevant literature and identifies the knowledge gaps.

Chapter 3 sets out the research methods in detail.

Chapter 4 reports on device reliability and moderating factors (published in 2016 and 2017).

Chapter 5 reports on cross-sectional analysis of the population of interest (published 2017).

Chapter 6 provides a descriptive analysis of the longitudinal data.

Chapter 7 completes the thesis with recommendations for the future.

Figure 1 is a graphic which will be used to track the progress of research activities through the thesis chapters.

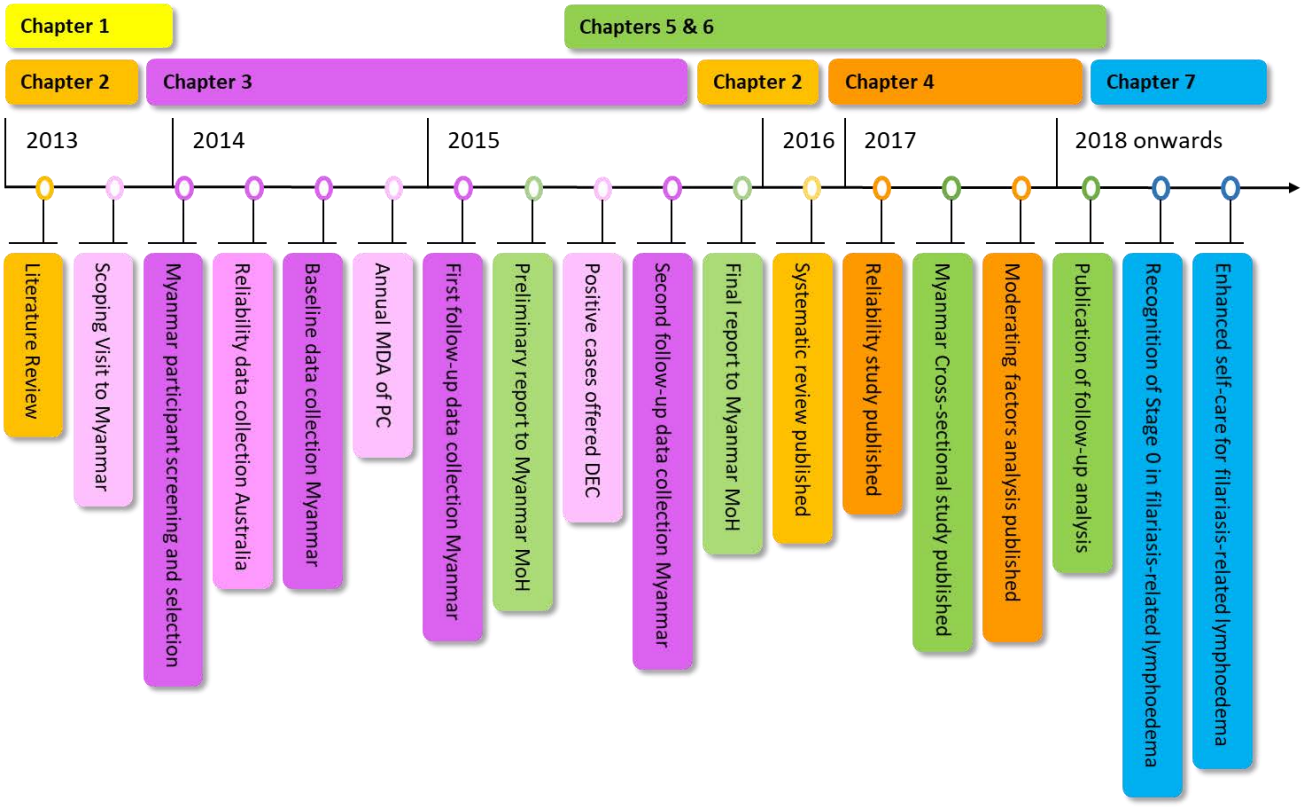


Figure 1: Time line of thesis activities and the thesis chapters in which they will be reported

1.1 Thesis Background

Lymphoedema is a chronic tissue disease which can lead to significant disfigurement and disability. Globally, the greatest cause is lymphatic filariasis (LF), a mosquito borne parasite endemic among many of the world's poorest people. Lymphoedema is not fatal, nor is hydrocele of the scrotum in males, another sequela of LF. While hydrocele can potentially be cured with surgical intervention, lymphoedema must be managed for life (WHO, 2013a). The most researched form is lymphoedema of the arm after breast-cancer therapy. In this population, active investigation to detect covert pathologic change and application of preventive intervention can prevent overt disease from developing (Stout-Gergich et al., 2008) and few women need progress to advanced stages.

A functioning lymphatic system returns fluid leaked from the capillaries into the tissue spaces back to the venous circulation. This nutrient rich extracellular fluid (ECF) also carries cellular waste and is drawn continuously through the tissue spaces by active pumping of the lymphatic vessels (Guyton & Hall, 2006). This micro-circulation; that is the passage of fluid and other elements from the blood capillary through the tissue spaces and into the initial lymph vessels, is fed by ultrafiltration at the capillary and drained by continuous pumping of the lymph collector vessels (M. Földi & Földi, 2012). An imbalance in the microcirculation can lead to oedema (swelling) in the subcutaneous compartment. If the imbalance is an excess capillary filtrate with normal lymphatic function, a low protein, dynamic-oedema will form. If capillary filtration is normal but lymphatic function is impaired a high protein, lymph-oedema will form (ISL, 2016).

There are multiple causes of lymphoedema, but whatever the cause, parasitic worm or treatment for breast cancer, lymphatic failure leads to the same chronic tissue disease. Despite this uniformity in pathogenesis, assessment techniques, staging criteria, and

treatment, guidelines for lymphoedema differ widely depending on the setting (Stout, Brantus, & Moffatt, 2012). At the highly resourced end of the spectrum, nuclear imaging and other sophisticated techniques such as bio-impedance spectroscopy (BIS) are often used to assess breast cancer-related lymphoedema (BCRL) of the arm (Case, Witte, Witte, Unger, & Williams, 1992; Cornish et al., 2001). At the low resourced end of the spectrum, assessment of filariasis related-lymphoedema (FRL) relies on grading of visible and palpable soft tissue changes (Ryan, 2004; Stout, Brantus, et al., 2012). There is no protocol to detect covert tissue disease or recognition of a latent stage in the FRL grading criteria. Without objective and sensitive ways to assess FRL, subtle but important improvements in tissue composition may be missed when treatment interventions are assessed (Wilson et al., 2004) and people at risk of developing FRL will not be offered preventive interventions.

Among the few neglected tropical diseases (NTD) with the potential to be eradicated, LF is also one of the most wide-spread with 70 million people already infected and another billion living in 73 tropical countries at risk of infection (Ramaiah & Ottesen, 2014). Considering both lymphoedema and hydrocele, the permanent disability caused by LF is second globally only to mood affective disorders (WHO, 1995) and estimates of the burden of LF morbidity in disability adjusted life years have increased 17.2% from 1990 to 2010 (Murray et al., 2012). Even greater public health impacts are likely if the effect on carers and communities is considered.

The Global Program to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000 with the goal of eliminating LF as a public health problem by 2020. Activities of the GPELF rest on the twin pillars of: preventive chemotherapy (PC) to interrupt disease transmission, and provision of health care services for people with manifest disease (WHO, 2010). To fulfil the first pillar, annual mass drug administration (MDA) of PC is delivered to all residents in

endemic regions. MDA activities may cease after five or six years if prevalence has dropped below the threshold required to sustain transmission. Morbidity management and disability prevention (MMDP), the activities of the second pillar, are more complex and require a patient-oriented approach with strategies ranging from surgical reduction of hydrocele to life-long home-based self-care for FRL (Mackenzie, Lazarus, Mwakitalu, Mwingira, & Malecela, 2009).

Countries which are validated by the World Health Organization (WHO) to have eliminated LF, have fulfilled the requirements of both pillars (WHO, 2017b) but are obliged to continue surveillance activities to detect and respond to any reappearance of the parasite. Similar monitoring of MMDP activities after PC has ceased is not stipulated, but it is not yet known if people with covert lymphatic disease will progress to filariasis-related lymphoedema (FRL) or have a complete reversal of covert tissue changes (Shenoy, Suma, Rajan, & Kumaraswami, 1998). Clarification of this unknown will assist endemic countries to plan for and deliver adequate services for all existing and at-risk FRL cases.

In a pilot study conducted in Papua New Guinea (PNG), Sue Gordon used tissue tonometry to detect changes in compressibility (compliance or stiffness of the skin and subcutis) on the lower-limbs of young LF-positive but asymptomatic cases. In the LF-positive group, skin over the posterior thigh was more compressible (softer) than among their uninfected peers and indicated the presence of covert tissue changes in the legs of the infected participants (Gordon, Melrose, Warner, Buttner, & Ward, 2011). These results generated the guiding questions for this thesis which is a further exploration of covert change in tissue composition in asymptomatic young people with LF.

The aim of this thesis was to contribute empirical evidence toward recognition and description of covert lymphoedema in GPELF related activities. A secondary aim was to

identify reliable, inexpensive, field-friendly devices that can detect clinically relevant changes in tissue composition associated with FRL.

1.1.1 Questions that guided the thesis

1. Can devices already used to quantify tissue changes in BCRL be used in LF endemic populations?
2. Can the devices of interest detect covert changes in asymptomatic LF antigen positive cases?
3. What is the effect of annual PC on tissue composition in LF antigen positive cases?

1.2 Aims

The aims of this thesis were:

1. To investigate the reliability of tissue tonometry to measure skin and subcutaneous tissue compressibility in the lower limbs of young people.

2. To investigate the reliability of bio-impedance spectroscopy (BIS) in measures of free fluid in the lower limbs of young people.

3. To determine if covert change in tissue composition occurs in the lower-limbs of asymptomatic LF-positive cases based on tissue tonometry and BIS measures.

4. To determine if the annual dose of preventive chemotherapy alters tissue compressibility and free fluid in LF antigen-positive cases.

1.3 Objectives

The objectives of this thesis were:

1.3.1 Preliminary studies

1. To review the current literature on LF-related lymphoedema.
2. To review current assessment techniques for secondary lymphoedema.
3. To conduct a reliability study on the devices of interest in Myanmar and Australia.

1.3.2 Main studies

4. To conduct a cross-sectional study on asymptomatic young people living in an LF endemic region in Myanmar.
5. To conduct a follow up study on the Myanmar cohort after MDA of PC.

1.4 Rationale for the thesis

Untreated lymphoedema follows a somewhat predictable course of pathologic changes in the skin and underlying tissue (Lawenda, Mondry, & Johnstone, 2009) initiated by a long-standing high-protein oedema in the epifascial compartment. Changes in tissue composition can be quantified by tissue tonometry which measures resistance to induration (compliance or stiffness) and fluctuations in the ECF can be measured in terms of electrical resistance using bio-impedance spectroscopy (BIS). In latent and very early stage lymphoedema, when a protein rich fluid is slowly accumulating in the tissue spaces, the skin remains soft to the touch and there is minimal, or transient, visible swelling. Pathological tissue changes are induced by trapped proteins and other inflammatory mediators (Nakamura, Radhakrishnan, Wong, & Rockson, 2009) and BIS scores indicate an increased extracellular fluid load while tonometry scores indicate increased tissue compliance (more compressibility).

In the middle stages when the subcutaneous compartment is visibly enlarged due to slowly accumulating fat and fibrous tissue, there is increased vascularisation and free fluid reduces as the connective tissue growth replaces the oedematous tissue spaces. Normal limb contours are lost, and the skin begins to take on an orange peel appearance. Tonometry scores will be lower and continue to fall as the tissue becomes stiffer over time. This stage is more difficult to treat and some tissue changes are now irreversible (Szuba & Rockson, 1998). Even so, the earlier symptoms are treated the more chance that progression can be reversed or at least halted. In later stages, the limb is grossly enlarged, skin is thick, and the underlying tissue becomes stiff with fibrous deposits. Tonometry values will be very low and since there may be little or no excess free fluid, BIS values may be close to normal. By this stage, the opportunity to reduce limb size through conservative therapies has been lost and management to halt progression is complex and relentless. Without effective strategies to

reduce limb size, the main aim of interventions for late stage FRL is to reduce the frequency and intensity of acute infections (Kerketta et al., 2005).

Given current estimates of more than 16 million people living with the daily burden of lymphoedema due to LF, there is a surprising lack of innovation in assessment or intervention when compared to recent advances in BCRL (Stout, Brantus, et al., 2012). Better evidence for the benefits of treatment and identification of covert FRL could lead to low-cost preventive strategies, but this requires objective assessment of clinically relevant changes using reliable devices. The mechanical Tonometer, well validated in research on BCRL and used in the pilot study, was able to detect covert changes among the asymptomatic LF-cases in PNG (Gordon et al., 2011). This device has several operational issues (see section 2.3.3.4) and an operator-friendly, electro-mechanical Indurometer was developed to overcome these and record resistance to the same tissue deformation parameters as the mechanical version (Pallotta et al., 2011). Indurometry requires no further consumables and may provide an objective and useful measure at the community or health clinic level. Assessment of free fluid (ECF resistance) can be accomplished by a portable battery-powered BIS unit, but the necessary use of disposable self-adhesive electrodes makes it more suitable for research settings than the rural health clinic.

For any group at-risk for any type of lymphoedema, it is unclear who will progress to overt disease or how long that will take. The most reported risk factor for BCRL is high BMI (>25kg/m^2) and onset may be years or even decades after cancer therapy is completed (Petrek, Senie, Peters, & Rosen, 2001). It is well demonstrated in the literature that early intervention in latent BCRL can prevent onset of overt disease (see Chapter 2 section 2.3.2). It is also well documented that familial factors and a long period of latency occur in those with morbidity from LF (Cuenco, Ottesen, Williams, Nutman, & Steel, 2009; Lammie, Cuenco, &

Punkosdy, 2002) and limited evidence that annual PC may reverse early stage FRL in children (Shenoy et al., 2009) hints that the potential exists for preventive intervention in FRL. It is proposed that recognition and inclusion of criteria for a latent or stage 0 FRL in WHO guidelines and research on preventive interventions may improve outcomes of MMDP activities under GPELF.

Chronic disease management includes: education in preventive behaviours in at-risk populations, monitoring and early intervention for high-risk cases, and access to services for known cases (Beaglehole et al., 2008). Recent trends in BCRL toward pre-operative and close post-surgical monitoring with preventive intervention at any sign of covert tissue change (Stout, Binkley, et al., 2012) will spare many breast-cancer survivors a life-time of daily self-management and increased health care costs. No such strategies exist within MMDP guidelines for FRL which is typically diagnosed at middle or later stages when the per-person cost of management is much higher (Stout, Brantus, et al., 2012). This resembles the BCRL scenario of more than two decades ago when understanding of BCRL and access to treatment were limited and many cases advanced to late stage disease. The logistics involved in implementing preventive strategies in resource poor settings are challenging but worth exploring. Economic modelling on outcomes from a self-care intervention among existing cases in India returned a per-person saving of more than 130 times the per-person cost of implementing the program (Stillwaggon, Sawers, Rout, Addiss, & Fox, 2016).

Intervention in BCRL has shifted paradigm frequently in the last 20 years and is now moving more strongly towards preventative strategies. It is expected that publications and post-doctoral work arising from this thesis will contribute to speeding up the inevitable advancement in FRL management toward earlier, population appropriate interventions and

contribute to establishing an appropriate surveillance period for MMDP activities to continue after LF transmission is interrupted.

1.5 Hypotheses for the cross-sectional and follow-up studies:

H1: Covert changes in tissue composition can be detected in asymptomatic, LF positive cases.

a) Infection with LF will lead to increased tissue compressibility in the lower limb(s). Three devices were used to test this aspect of the hypothesis:
    - A mechanical Tonometer (SA Biomedical Engineering, Australia)
    - An electro-mechanical Indurometer (SA Biomedical Engineering, Australia)
    - A SkinFibroMeter (Delfin Technologies, Finland)
b) Infection with LF will lead to increased free fluid in the lower limb(s). One device was used to test this aspect of the hypothesis-
    - A portable BIS unit, the SBF7 (Impedimed, Australia)

H2: Covert changes in tissue composition detected in asymptomatic LF positive cases are reversed after consumption of PC during annual MDA.

a) Annual MDA of PC will change tissue compressibility in the lower limb(s) of LF positive cases. All three devices were used to test this aspect of the hypothesis but only one device, which had found significant between-infection-group differences at baseline, was included in the follow-up analysis-
    - Indurometer (SA Biomedical Engineering, Australia)
b) Annual MDA of PC will reduce excess free fluid in the lower limb(s) of LF positive cases. One device was used to test this aspect of the hypothesis-
    - SBF7 (Impedimed, Australia)

1.6 Study design

A memorandum of understanding (MOU) between James Cook University (JCU) and the Myanmar Ministry of Health and Sports (MOHS) was exchanged in 2015 and the study was conducted in accordance with that memorandum. Appendix A1 is a copy of the MOU.

The study was designed in three parts.

1. A cross-sectional study using the devices of interest in the lower limbs of healthy young people residing in Myanmar and Australia. Outcomes were to:
    a. Establish intra-rater reliability of the devices.
    b. Identify moderating factors associated with variance in measures.
2. A cross-sectional study on age and gender matched young people living in a filariasis endemic region in central Myanmar. Outcomes were to:
    a. Determine if covert changes in tissue composition occur in LF positive cases.
3. A follow up of participants present at baseline after administration of PC via MDA. Outcomes were to:
    a. Determine if covert changes in LF-positive cases detected at baseline were altered after consumption of PC.

1.6.1 Amendments to the study design

First follow-up was six weeks after the 2014 MDA, and preliminary analysis revealed a low level of PC consumption among study participants (40%). This was much less than the level of coverage reported by the Myanmar LF elimination program and the > 65% coverage required to interrupt transmission. The Myanmar MOHS was notified and the

positive cases who had not received PC during the December 2014 MDA were offered a 12 days course of daily diethylcarbamazine citrate (DEC) during March 2015. PC during the MDA was a single dose each of Albendazole and DEC. The low PC consumption during the MDA had also resulted in small groups among the antigen-positive cases for the before-and-after PC analysis, therefore a further follow-up was conducted six weeks after the additional PC had been offered.

A randomised self-care intervention on antigen-positive cases found to have any sign of covert FRL was planned and a systematic review on self-care for secondary lymphoedema was conducted. Due to time constraints, this intervention could not be included in the final study design. Results of the systematic review informed data collection methods, data analysis, and interpretation throughout the remaining research activities.

1.7 Methodology overview

The study was a before and after comparative cohort study on young people in a filariasis endemic population. A schematic overview of the objectives and research activities of the thesis can be seen in Figure 1.7.

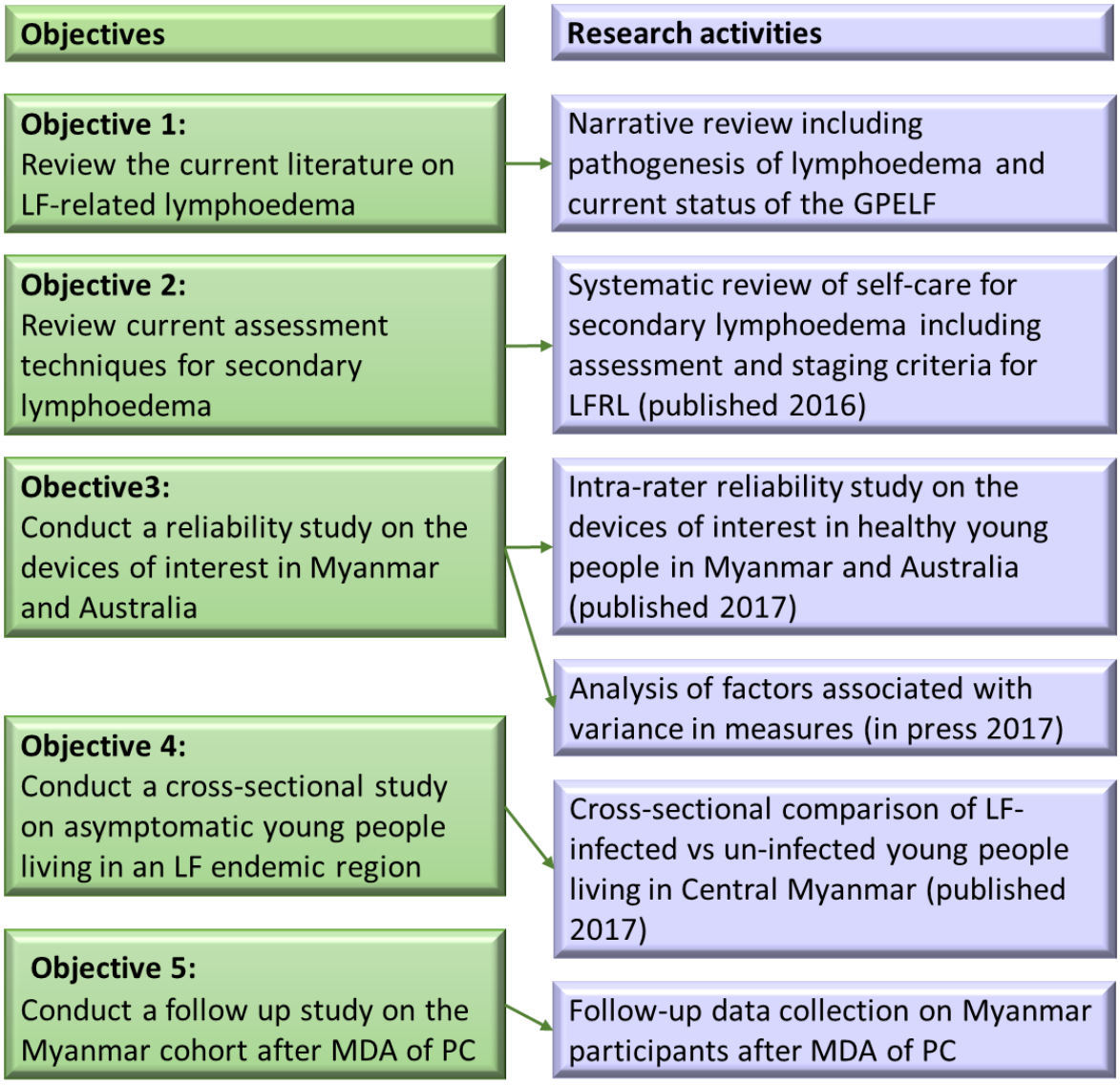


Figure 1.7: Schematic overview of thesis development "Covert tissue changes in lymphatic filariasis and implications for the future of morbidity management"

1.7.1 Narrative and systematic literature reviews

Chapter 2 is a review of the published literature and includes: a narrative review of the history of lymphatic filariasis, pathogenesis of tissue disease following lymphatic failure, current approaches to differential diagnosis, and treatment. A systematic review on self-care for secondary lymphoedema explored similarities and differences between BCRL and FRL to identify transferable strategies in assessment and management. The reviews are drawn together to highlight the evidence for early intervention and preventive strategies.

The systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Liberati et al., 2009) and was published in PLOS-NTD in 2016 (Douglass, Graves, & Gordon, 2016). Portions of the systematic review are reported in Chapter 2 and the full publication is available in Appendix F1.

1.7.2 Data collection

Chapter 3 provides data collection methods for all studies including:

- Manufacturer specifications for each device and a detailed description of their operation in the study.
- Ethical approvals in Australia and Myanmar.
- Recruitment and participant consent processes in each location.
- Data collection protocols.
- Statistical tests performed on the data.

1.7.2.1 Analysis of reliability and moderating factors in the devices of interest

Since none of the devices investigated are routinely used to assess the lower limb, or young people, or FRL, a reliability analysis was required and performed. Data on uninfected cases in the Myanmar cohort and a similarly aged cohort of young Australian people were used to determine: the intra-class correlation coefficient (ICC) for each device and the level of the agreement (coefficient of variation) between devices for each measurement point. Stepwise linear regression was undertaken to determine factors related to variance in the measures in each population such as age, gender, and limb dominance.

Results from the reliability analysis and the report on factors association with variance in device measure are presented in Chapter 4. Both studies were published in Lymphatic Research and Biology in 2017 (Douglass, Graves, & Gordon, 2017a, 2017b) and full text publications are available in Appendices F2 and F3.

1.7.2.2 Comparison of LF-infected and uninfected cases and the effect of PC

Data were collected with all devices at three time-points and baseline results for all devices were reported in the cross-sectional analysis of LF-positive and negative cases. The longitudinal analysis focussed on the effects of PC within each infection group and included only devices that had detected significant differences at baseline. All data have been uploaded to the JCU data repository (Douglass, 2017).

Baseline data were collected during October 2014 and the results of the cross-sectional analysis (Chapter 5) were published in the journal: Tropical Medicine and Infectious Disease, in a Special Issue "Neglected and Emerging Tropical Diseases in South and Southeast Asia and Northern Australia", in 2017 (Douglass, Graves, Lindsay, et al., 2017). A full-text of the publication is available in Appendix F4.

Follow-up data were collected in February and June 2015 and descriptive results of the longitudinal study are included in Chapter 6. A manuscript is being prepared for publication in 2018 after further statistical modelling has been completed.

1.8 Study outputs and importance of findings to the GPELF

As many counties approach the end of MDA, attention turns to providing services for existing cases to fulfil the requirements of the WHO validation dossier. The timely introduction of reliable measurement devices and the other insights arising from this thesis have potential applications in operational research on- and enhancement of- MMDP activities within the GPELF. A list of thesis outputs and their potential contribution can be seen in Figure 1.8.

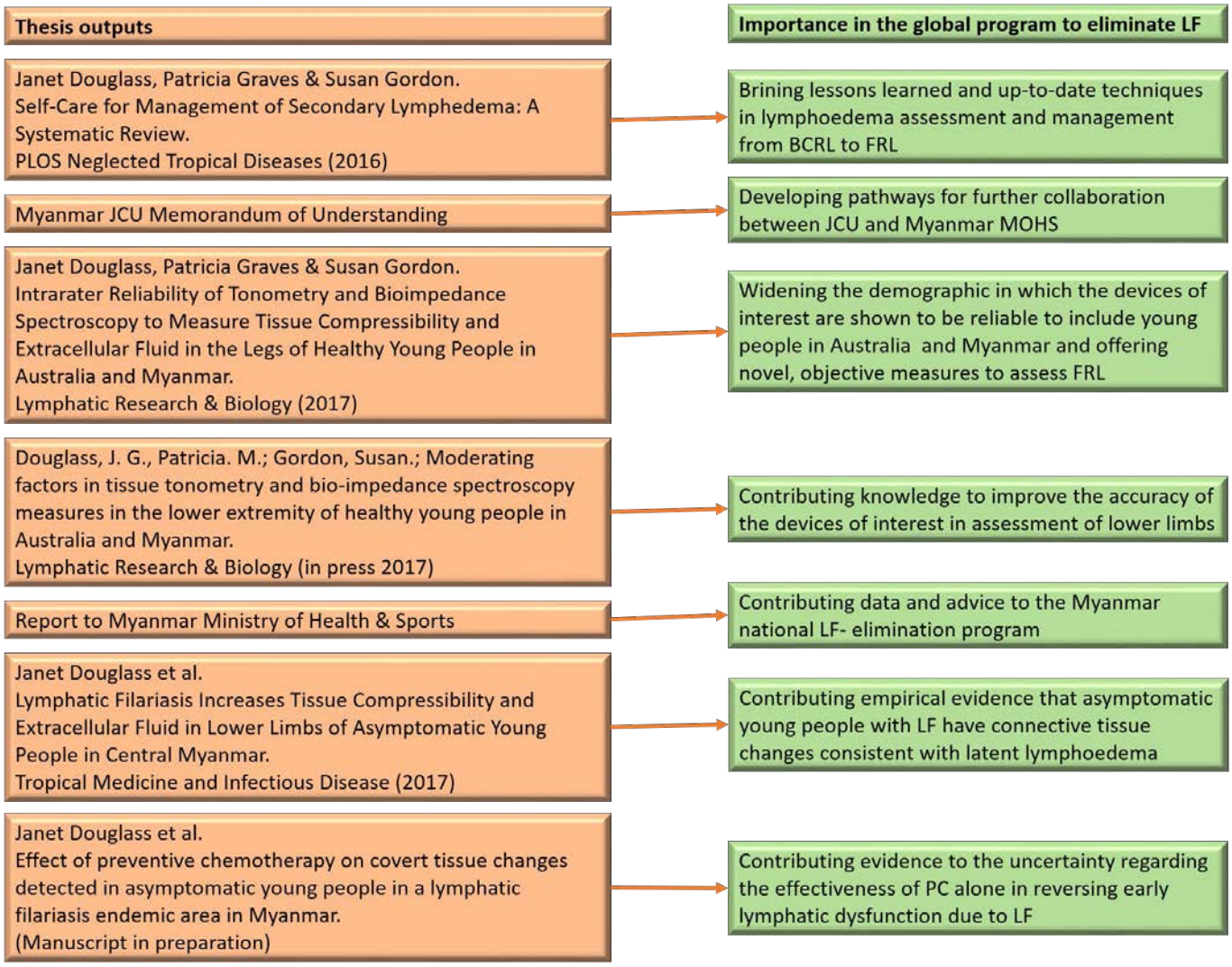


Figure 1.8: Study outputs and importance in the global program to eliminate lymphatic filariasis

1.9 Key points

1. Transmission of new cases of LF can be interrupted with successful PC via annual MDA, but existing and future cases of FRL will require lifelong MMDP services.

2. Best practice among breast cancer patients at risk for BCRL is detection of covert changes and early intervention to prevent onset of overt disease.

3. MMDP guidelines for FRL are lacking preventative interventions and offer few strategies to reverse early stages.

4. The effect of PC on covert tissue changes is unclear.

5. Early results from a pilot study in PNG indicate that tissue compressibility increases in asymptomatic LF-positive cases.

6. Inexpensive, field-friendly devices which can detect subtle changes in lymphatic function and objectively measure change in FRL status are needed.

7. Devices used to objectively assess BCRL are untested in FRL populations.

Chapter 2: Literature review

Chapter 2 provides a narrative review of the literature which informed the study design and methodology, and was used to form the questions in the systematic review on self-care in lymphoedema. Figure 2 shows the research activities for this chapter. The systematic review was published in 2016 in PLOS-NTD (Douglass et al., 2016).

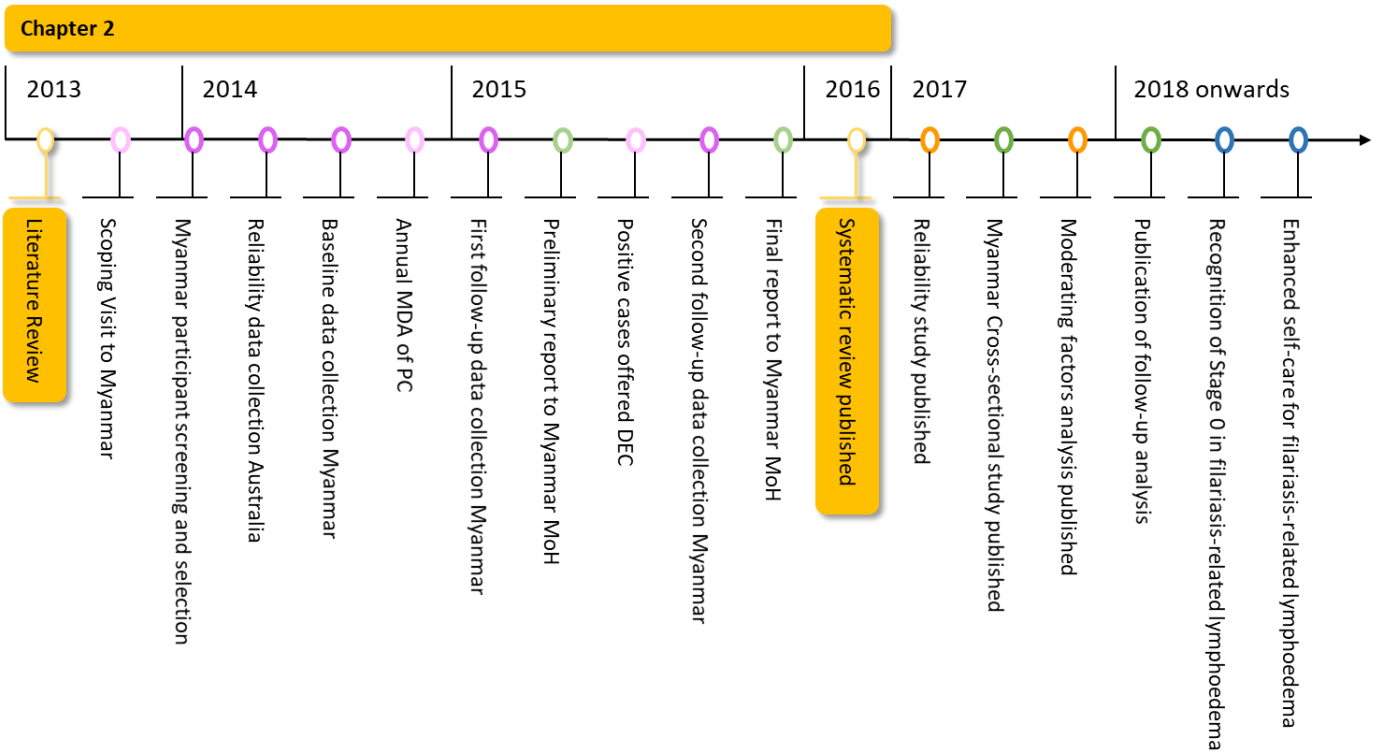


Figure 2: Research activities related to the narrative and systematic literature reviews

2.1 A brief history of lymphatic filariasis

Lymphoedema and hydrocele have been present in human populations for millennia. Statues with swollen limbs in the Nile region and images depicting scrotal swelling in Western Africa date back to 2000BC and 500BC respectively. As summarised by Cox (2002), ancient Chinese and Indian physicians described the disease and ancient Greek and Roman literature offer differential diagnosis of elephantiasis (advanced lymphoedema) from leprosy. The first written record of an LF affected population appears in the late 16th century from the Dutch explorer Jan Huyghen van Linschoten who wrote that the inhabitants of Goa were *"all born with one of their legs and one foot from the knee downwards as thick as an elephant's leg"* (van Linschoten, 1885). Reports from Asia and Africa soon followed. In the latter half of the 19th century, following discovery of microfilariae; in fluid extracted from a hydrocele (French surgeon Jean-Nicholas Demarquay 1863), in urine (Otto Henry Wucherer 1867), and in blood (1872), a British Physician, Timothy Lewis, made the connection between microfilariae and elephantiasis. Soon after, Joseph Bancroft described the adult worm now recognised as *Wuchereria bancrofti* (1886). An understanding of the worm life-cycle was completed by Patrick Manson in 1877 who made the connection to the mosquito as intermediary host and vector of transmission (Otsuji, 2011). This was the first time an arthropod was identified as a disease vector and the resulting paradigm shift in parasitology led to discoveries in other tropical diseases such as malaria. But Manson was wrong about the means of transmission, which he believed to be via drinking water contaminated with microfilariae by mosquitos. In 1900, George Carmichael Low discovered microfilariae in the proboscis of mosquitoes and finally pinpointed the true mechanism of transmission (Cox, 2002).

Throughout the 20th century, research on epidemiology, aetiology, pathogenesis, and strategies to interrupt LF transmission was prolific and robust. Three worm species were

found to cause lymphatic filariasis in humans. *Brugia timori* and *Brugia malayi,* as their taxonomy suggests, are endemic to regions in South East Asia. *W. bancrofti* causes most of the infections world-wide and is endemic in tropical regions throughout the Western Pacific, Asia, and Africa (Melrose, 2002; Michael, Bundy, & Grenfell, 1996). Microfilariae are transmitted by several mosquito species depending on the geographical location and the worm. *W. bancrofti* can be transmitted by mosquito species of the genus *Culex, Anopheles,* and *Aedes*. In rural areas of Africa and the Americas, *Anopheles* are the most common vector whereas in urban and semi-urban areas of Asia, *Culex quinquefasciatus* is the vector. This diversity in worm- and vector-species presents complex challenges in approaching LF elimination from a purely vector-control perspective (Ottesen, 2006).

2.1.1 The global program to eliminate lymphatic filariasis (GPELF)

Eradication, the complete global extermination of a pathogen with no chance of reintroduction, has been achieved only for smallpox (humans) and rinderpest (cattle). The term 'elimination as a public health problem' is defined as: cessation of transmission in a country, continent, or other limited geographic area and, complete prevention of clinical presentation of a disease (WHO, 2015a). Once elimination is achieved, continued surveillance is required to ensure infection rates remain too low to support transmission, and ongoing monitoring is needed to prevent reintroduction of infection from other areas. The process of documenting elimination as a public health problem is called validation (WHO, 2017b).

Of the 20 NTDs affecting many developing populations, eight are considered to have the potential to be eliminated with current knowledge and technology. Lymphatic filariasis can be included in this group as there is no non-human reservoir and there are safe and affordable medicines available. The discovery of one of the drugs, the filaricidal agent avermectin (now known as Ivermectin) in 1975 by Satoshi Ōmura and William C. Campbell,

paved the way for the development of the current program to eliminate LF and Ōmura and Campbell were awarded a Nobel prize for the discovery in 2015 (Campbell, Burg, Fisher, & Dybas, 1984; Molyneux & Taylor, 2015; Tambo, Khater, Chen, Bergquist, & Zhou, 2015).

The first 'Informal Consultation on Evaluation of Morbidity in Lymphatic Filariasis', a meeting of experts in tropical diseases, was held in Madras in 1992 (WHO, 1992) and in 1997 the Fifth World Health Assembly passed resolution WHA50.29, *Elimination of lymphatic filariasis as a public health problem* (WHO, 1997). The Global Program to Eliminate Lymphatic Filariasis (GPELF) by 2020 was launched in 2000 (WHO, 1999, 2015b) with two main objectives often referred to as 'twin pillars'. One pillar is aimed at interruption of disease transmission though preventive chemotherapy (PC) and the other pillar provides a minimum package of care for people with existing morbidity. Interrupting transmission of the microfilariae is achieved via mass drug administration (MDA) of the PC in endemic regions (various combinations of Albendazole, Ivermectin or diethylcarbamazine citrate (DEC) depending on the location and co-endemic diseases). Albendazole and DEC are used in most countries and supplies in the GPELF are donated by manufacturers. At least five contiguous rounds of annual MDA are required to reduce infection rates to levels that cannot sustain transmission. This is because the PC agents are microfilaricidal but do not completely kill adult worms which may recover and remain reproductive. Therefore PC needs to be continued with adequate population coverage (at least 65%) until all existing worms have died (natural life span of the worm is 4 – 6 years) (Ottesen, Duke, Karam, & Behbehani, 1997).

As with other mosquito borne diseases, vector control can be an effective method to interrupt transmission. For example, indoor spraying with DDT (dichloro-diphenyl-trichloroethane) is thought to have been responsible for LF elimination in the Solomon Islands, (Bockarie, Pedersen, White, & Michael, 2009) and the use of pyrethroid-impregnated bed nets has

contributed to reducing parasite transmission by *Anopheles* and *Mansonia* mosquitoes. China used a combination of MDA with DEC and the supply of table salt containing 0.3% of DEC in endemic regions and achieved elimination of LF in 2006 (De-jian, Xu-li, & Ji-hui, 2013). Countries participating in the GPELF may employ any combination of methods to interrupt transmission of LF (Bockarie et al., 2009; Dickson et al., 2015). Of the original 83 countries identified as endemic for LF, 73 are involved in the global elimination program. Of these, 20 no longer require MDA but six countries have not yet commenced any MDA and 16 have started but not implemented MDA in all areas (implementations units) (WHO, 2017a). Countries that have now validated elimination as a public health problem include Togo, Maldives, Sri Lanka, Cambodia, Cook Islands, Marshall Islands, Niue, Tonga and Vanuatu (WHO, 2017a).

The second pillar, providing health care for people with chronic disease, is achieved through morbidity management and disability prevention (MMDP) services which involve provision of hydrocele surgery and a minimum package of care for lymphoedema (WHO, 2010). Although referred to as the 'twin pillars' MDA and MMDP are rarely applied equally, with MDA preceding MMDP by many years in most countries (Addiss & Brady, 2007; Cantey, Rout, Rao, Williamson, & Fox, 2010). Nevertheless, countries must demonstrate successful implementation of both pillars to be declared LF free and countries which have focused mainly on the MDA will need to start, or significantly increase, MMDP activities to meet the 2020 global targets (WHO, 2015b). The current lag between MDA and MMDP can be attributed, in part, to the complexity involved in implementing MMDP and the lack of operational research on country specific requirements and expected benefits (Kumari, Yuvaraj, & Das, 2012; Narahari et al., 2017). To properly implement and assess MMDP programs at a national level, objective measures of tissue changes associated with LF are needed, but collection of empirical data in resource poor settings has been hampered by the

lack of field-friendly, universally available yet sensitive assessment tools (Douglass et al., 2016).

2.1.2 GPELF in Myanmar

All mainland Southeast Asia countries are endemic for LF and in Myanmar this includes 45 of the 64 districts. The central western 'dry zone' is highly endemic with pre-MDA prevalence estimates of 20-30%. In 2011 the Myanmar national elimination program reported to the Regional Program Review Group (RPRG) that mapping activities were completed nationally in 2007 with over 46 million people living in endemic areas. MDA commenced in one district in 2001 but there were interruptions in 2005 and 2008 and in 2010 only 26.7% of endemic areas were covered due to issues with drug supply (WHO, 2011). The mean national prevalence has reduced from 7.1% in 2001 to 2.7% in 2011 but with inconsistent MDA coverage in some areas (Dickson, Graves, & McBride, 2017). The report to the WHO regional program review group in 2016 indicated that MDA was delivered in 38 of 47 endemic units in 2015, but that less than half of all units had received more than 5 rounds (20/27) (Tun, 2016). A morbidity survey in Mandalay Region also found low rates of self-reported PC consumption. Of the six rounds of MDA delivered between 2001 and 2015 in that region people reported taking PC an average of less than three times (Dickson et al., 2015). Information on LF morbidity in Myanmar is also patchy but the significant morbidity found in Myanmar migrants in Thailand (8.6% - 16.1%) suggests that the prevalence rate may be much higher than Myanmar government estimates (0.19% - 0.59%) (Dickson et al., 2017).

2.2 Lymphoedema

Lymphoedema is a symptom, and the causes of lymphoedema are numerous. Familial factors which lead to congenital malformation of the lymphatic vasculature or its function are termed primary lymphoedema. Secondary lymphoedema arises after disruption to an otherwise normally formed and functioning lymphatic system (ISL, 2016). Pathogenesis of lymphoedema from any cause will usually follow specific tissue changes which arise because of failure in lymphatic transport and will appear distal to the area of damage or lost lymphatic function. For example, BCRL will likely appear in whichever lymphatic territory of the hand or arm was previously drained by the axillary lymph nodes that were removed during surgery (Lawenda et al., 2009). Lymphoedema arising from LF mostly frequently appears in the distal leg as a result of worm nests formed near the inguinal lymph nodes, but can also occur in the arms, breast, and genitals (Dreyer, Noroes, Figueredo-Silva, & Piessens, 2000). Regardless of the cause, lymphoedema frequently begins with a long period of latency and it is well known that most children infected with LF will remain asymptomatic, often until young adulthood (Mandal, Bal, Das, Achary, & Kar, 2010). Although most arm lymphoedema after breast cancer will appear within three years of the original surgical intervention, over one in five cases will have late onset BCRL (Petrek et al., 2001) and a latent phase of up to 30 years has been described in some cases (Brennan & Weitz, 1992; Lawenda et al., 2009).

2.2.1 Lymphatic function

Lymph collector vessels are the main transport mechanism of the lymphatic system. Lymph is moved through the vessels by contraction of specialised smooth muscle layers in the vessel wall and directed towards the regional lymph nodes by internal valves. Lymphatic vessel pumping is continuous and controlled by several mechanisms (Zawieja, 2009). Stretch-receptors within the vessel walls and chemokines within the lymph stimulate contraction of

the smooth muscle in the vessel wall and in this way the system can increase or decrease the rate of lymph pumping in response to the lymphatic load and contents (Mislin, 1961). Even when there is not enough lymph within the vessels to initiate contraction, the specialised smooth muscle is auto-myogenic and will contract at fixed intervals to prevent lymph stasis (Olszewski & Engeset, 1980). The lymphatic system works in conjunction with the cardiovascular system to maintain the correct level of fluid, circulating proteins, and immune elements within the loose connective tissue which is the medium though which all cells receive their nutrient supply and expel their wastes. A few waste products such as carbon dioxide are returned to the capillary by diffusion, but the bulk of the ultrafiltrate will pass through the interstitial spaces and exit via the initial lymph plexus, along with any invading microorganisms or other harmful substances that may have entered the connective tissue spaces. Once inside the lymphatic vasculature, the fluid now called lymph, is passed through a series of lymph nodes where some water is returned to the venous circulation and constituents of the lymph that could be harmful are destroyed by immune cells (Jafarnejad, Woodruff, Zawieja, Carroll, & Moore Jr, 2015). The cleaned and filtered lymph is transported through the remaining lymphatic vasculature until it reaches the thoracic- and right-lymphatic ducts which empty into the respective subclavian veins (Guyton & Hall, 2006).

The superficial and deep lymphatic systems, whilst connected, operate in different environments and the density of vessels and degree of lymphatic pumping varies depending on whether they are located in the superficial or deep compartments of the body (Lawenda et al., 2009). The deep system, that is everything below the tough elastic stocking that coats the musculature (deep fascia), is in a high-pressure environment where skeletal muscle activity, fascial compression forces, and even the pulsation of arteries within the perivascular sheath help to promote lymph flow. In this compressed environment, small blood vessels and cells are held in close proximity to each other, and oedema of any kind rarely forms.

In the superficial system, above the deep fascia, the skin does not apply much external compression and in this compartment, the suction forces created by lymphatic pumping reduce the interstitial pressure to slightly less than atmospheric pressure (Aukland & Reed, 1993; Guyton, Scheel, & Murphree, 1966). Atmospheric pressure then, creates the necessary external compression required to maintain the close association between capillaries and cells in the skin and epifascial compartment. If the ECF volume or pressure increases in the subcutaneous compartment, lymphatic pumping increases in rate and force and the interstitial spaces do not expand. This differential between the interstitial and atmospheric pressures is responsible for the appearance of body contours such as superficial veins and the hollows behind the ankle. Lymphatic pumping is slow and continuous, and the low-pressure state of the subcutaneous compartment is easily maintained using only 10-20% of maximum lymph transport capacity. The superficial lymphatics also contribute to barrier defences by continuous removal of invading microorganisms or other harmful substances via the initial lymph plexus which is located immediately under the skin (Sherwood, 2015).

2.2.2 Oedema formation and pathogenesis of lymphoedema

The cellular and cardiovascular compartments of the body are tightly regulated, and fluid and protein levels do not vary greatly within each of these closed compartments. The connective tissue spaces on the other hand are open compartments where large glycosaminoglycan molecules such as hyaluronan can hold or release water as needed (Villeco, 2012; Wiig & Swartz, 2012). If viewed as a connective tissue 'bath', then capillary filtrate acts like the tap, providing nutrient rich fluid to replenish the bath water. The function of the drain is provided by the openly porous initial lymph plexus and lymphatic pumping in the proximal vessels. If lymphatic transport capacity is reduced below that required to remove the lymph obligatory load, elements such as circulating proteins which are normally removed on a continuous

basis, may begin to accumulate in the tissue spaces (Mortimer & Rockson, 2014). If the volume or pressure of the fluid remains below atmospheric pressure no visible swelling will be seen. If the volume or pressure of fluid becomes greater than atmospheric pressure, superficial contours will be lost, and the oedema will appear as a visible swelling. In the early stages swelling may be transient, disappearing on elevation or overnight rest. The skin still looks and feels normal but the subcutaneous compartment is slowly flooding and local inflammatory processes are triggered by the accumulating and degrading proteins (Szuba & Rockson, 1997).

As the swelling becomes apparent, trapped proteins make the fluid thixotropic (gelatinous) and a deep and persistent pit is easily made with thumb pressure into the skin (pitting) (Bagheri, Ohlin, Olsson, & Brorson, 2005), but the chronic tissue disease that characterises lymphoedema is more than a simple overload of free fluid in the tissue spaces. Pathological changes in the tissue architecture occur over time, excess free fluid is replaced by fat and fibrous tissue, there is increased vascularisation, and overall free fluid reduces (Zaleska & Olszewski, 2017). The swelling no longer diminishes on elevation or overnight rest and only shallow pits can be made. The subcutaneous compartment may become grossly enlarged and the opportunity to significantly reduce limb volume through conservative therapies has passed. In advanced stages the skin also thickens (hyperkeratosis), the underlying tissue feels hard on palpation (fibrotic), and the affected body part takes on the characteristic appearance which inspired the eponym ‘elephantiasis‘ (Szuba & Rockson, 1997). Interruption to normal lymph flow also hampers immune responses as normal circulation of immune elements is dependent on lymph flow. In advanced cases, the frequency and severity of secondary infections often engulf all primary resources and the only outcome that is actively pursued is the reduction in frequency of acute episodes (Deng et al., 2015).

2.2.2.1 LF-related lymphoedema

Despite the extent of research and resources expended towards identifying filarial worms and their vectors, relatively little is known about the earliest tissue changes that occur before clinical symptoms of lymphoedema can be seen, or which factors determine progression from asymptomatic infection to chronic morbidity (Addiss & Brady, 2007). Larval worms (microfilariae) are injected beneath the skin by an infected mosquito during a blood meal, but like all invading microorganisms, the microfilariae are immediately removed into the initial lymph plexus and transported toward the lymph nodes where many are eliminated by normal body defences. Worms that escape immunological defences reside in dilated segments of the lymph vessels proximal to lymph nodes which are referred to as worm nests. The worms reach reproductive maturity in 6 – 8 months, mate, and release millions of microfilariae into the blood for up to ten years (Chakraborty, Gurusamy, Zawieja, & Muthuchamy, 2013). Lymphatic filariasis has a very low infectivity rate and estimates that 15,500 infective bites or more are necessary to produce one new case of microfilaremia have been postulated (Hairston & de Meillon, 1968).

People with circulating microfilaria are the source of transmission to others and global estimates put current cases of infection at close to 70 million with 36 million microfilaria carriers. Close to 20 million men are estimated to have filariasis-related hydrocele and as many as 17 million people are living with filariasis-related lymphoedema (Ramaiah & Ottesen, 2014). This suggests that less than 25% of infected cases will progress to manifest lymphoedema, which is lower than estimates of the incidence of lower limb lymphoedema after gynaecological cancer or melanoma which can range up to 50% (A. F. Williams, Franks, & Moffatt, 2005). The risk factors which determine who will develop overt lymphoedema are complex and include genetic or familial factors (Cuenco et al., 2009) and persons with overt FRL may no longer host active parasites.

PC alone provides no further benefit in advanced disease (Shenoy et al., 1998) and any lymph vessel damage sustained during infection may be permanent, leading to lymphoedema in the absence of live worms (Nutman, 2013). Unlike BCRL where parts of the lymphatic system are surgically removed or irreparably scarred, the damage to lymphatic vasculature in LF arises from sustained inflammatory processes that have a progressively harmful effect on lymphatic function (Nutman, 2013). Acute episodes of adeno-dermato-lymphangitis (ADLA) exacerbate lymphatic damage, contribute to disease progression and can occur with or without covert sign of lymphoedema (Olszewski et al., 1999).

While the majority of people with LF will have no overt sign of lymphatic disease, there is evidence that all adult worm carriers have some level of lymphangiectasis (pathologic vessel dilation) (Dreyer et al., 2000) and subclinical damage might have already occurred in limbs that appear normal (Wilson et al., 2004). However there has been no way to determine which of all infected individuals will progress to overt disease. Manifestation of clinical symptoms depends on multiple contributing factors including worm species, geographical location, natural residence, gender, genetics, and personal risk of infection but these factors are not yet fully understood (Addiss et al., 2010; Cuenco et al., 2009).

The biochemical cascade in the connective tissues that leads to lymphoedema is still a largely unexplored landscape and recent discoveries in the interaction between filarial worms and human immunological defences indicate that the inflammatory stimulus may not be worm antigen but endosymbiotic *Wolbachi* bacteria which are essential to worm reproduction (Taylor, Cross, & Bilo, 2000). Efforts are now underway to exploit this knowledge in terms of improved PC regimes (Irvine et al., 2017) and the potential role of anti-inflammatory or antibiotic therapy in reversing early lymphoedema (Debrah et al., 2006).

2.3 Assessment of lymphoedema status

People living with lymphoedema face daily challenges. Sufferers of FRL and their families are faced with lifelong daily management, reduced quality of life or opportunity to marry, and with increased health care costs (Chandrasena, Premaratna, Muthugala, Pathmeswaran, & de Silva, 2007). People with BCRL also report depression, poor quality of life, and an inability to engage in paid employment (Ridner, Dietrich, & Kidd, 2011). Psycho-social and economic impacts in lymphoedema are under-explored (Zeldenryk, Gray, Speare, Gordon, & Melrose, 2011) but can be evaluated and are important in guiding appropriate provision of resources and lymphoedema services (Shih et al., 2009). For the purposes of this literature review, only methods to evaluate physical parameters of lymphoedema status have been included such as: stage of lymphoedema progression, limb enlargement, excess free fluid, and changes in skin and underlying tissue tonicity. The focus will be on methods which are suitable for use in developing country settings by local health workers and therefore does not include lymphatic imaging techniques such as lymphoscintigraphy which require expensive, non-portable medical equipment and tertiary qualified operators.

2.3.1 Lymphoedema stage

Clinically manifest lymphoedema follows a somewhat predictable course of changes in the skin and underlying tissue, and key features such as the presence of skin folds or papillomatosis can be used to grade lymphoedema as it passes through defined stages. A variety of grading or staging criteria are used and the lack of any universally accepted system limited the use of meta-analysis in two systematic reviews on FRL (Douglass et al., 2016; Stocks, Freeman, & Addiss, 2015).

Staging criteria from two main agencies are the most commonly used depending on the setting. In developed country settings a three-stage system, described by the International Society of Lymphology (ISL) in a consensus document on diagnosis and treatment of peripheral lymphoedema (ISL, 2016), is frequently used in assessment of BCRL of the arm. Plate 2.3.1.1 provides pictorial examples of Stages 1 – 3 and Table 2.3.1.1 shows the ISL criteria and treatment recommendations. The signs and symptoms within each stage are very broad, so Stage 2 may be further divided into Stage 2a and 2b. A latent stage is recognised as necessary, and a Stage 1a is frequently referred to, but since the criteria are not well defined has not yet been formally added to the consensus document (see section 2.3.2).

In developing countries the seven-stage system described by Dreyer and colleagues (2002) is the current standard used in WHO training materials for LF program managers (WHO, 2013a) although previous versions with three and four stages are still occasionally used (WHO, 1985, 1992). WHO guidelines used in the South-east Asia regions use three stages (WHO, 2013b). Plate 2.3.1.2 shows the seven stage criteria with pictorial examples and Plate 2.3.1.3 provides the treatment recommendations for each of the seven stages.

As well as the large diversity between these two staging systems, researchers and clinicians report inconsistencies in classification within each system (Cheville et al., 2003; Logan, 1995; Stocks et al., 2015; Vanderstelt et al., 2015). When the seven stage criteria are used the stages are often grouped into mild (stages 1 – 2), moderate (stage 3) or severe (stage 4 – 7) for statistical analysis. The results from the systematic review concluded that these graduations may lack the precision to detect small changes occurring as a result of basic home-care interventions (see section 2.4.1.7) (Douglass et al., 2016).

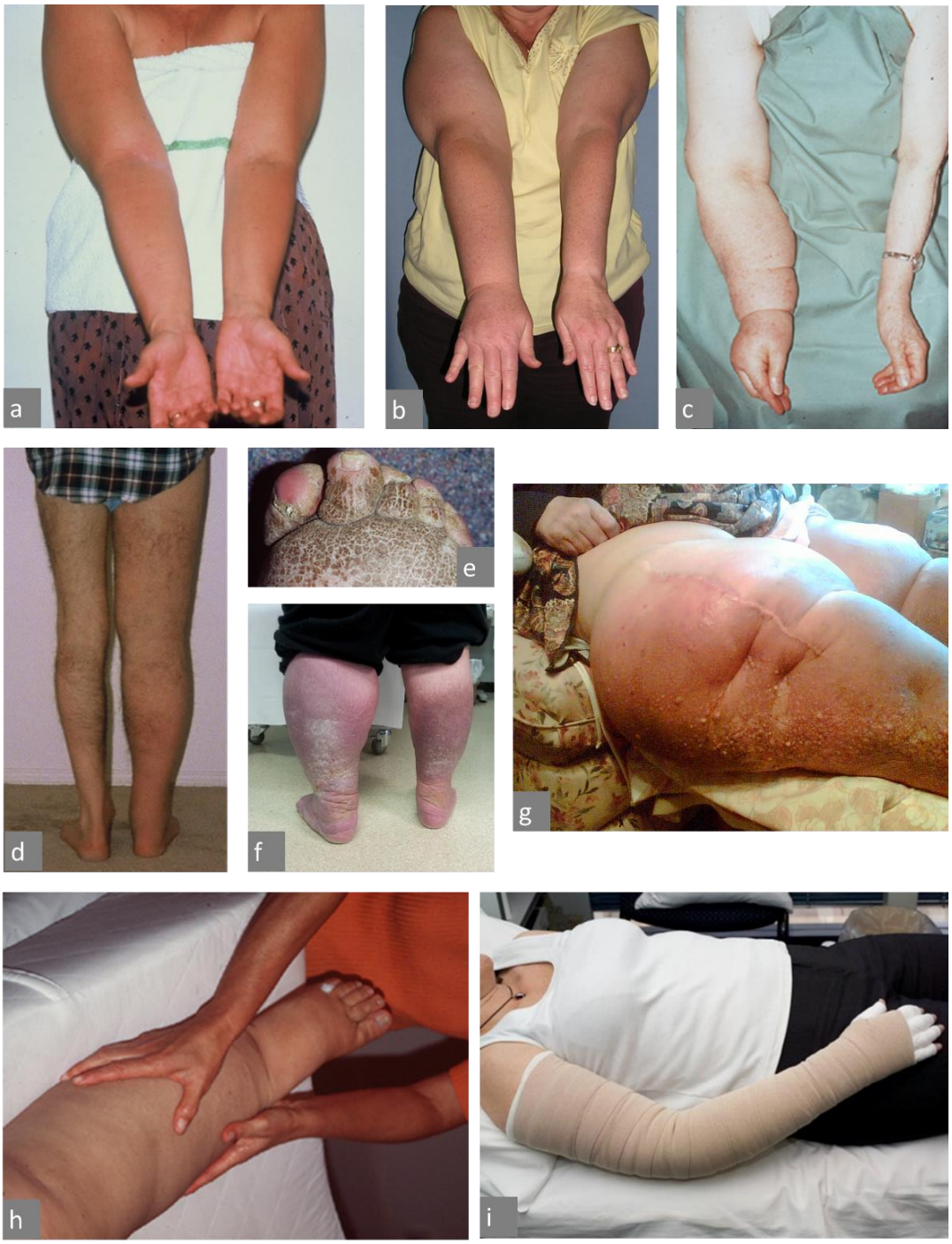


Plate 2.3.1.1: ISL stages of lymphoedema a) Stage 1 breast cancer-related lymphoedema (BCRL) of the arm, b) Stage 2a BCRL of the arm, c) Stage 2b BCRL of the arm, d) Stage 2b primary lymphoedema of the lower-limb, e) Stage 3 lymphoedema with hyperkeratosis of the forefoot and toes, f) Stage 3 lymph-oedema of the leg with shallow skin-folds, g) Stage 3 lymphoedema of the lower-limb with lymphoceles, h) Therapist performing lymphatic massage, i) multi-layer compression bandaging. Photographs b), c), e), f), g), h) & i) are courtesy of Professor Neil Piller and photographs a) and d) are courtesy Robert Harris and Linda Blanchfield respectively.

Table 2.3.1.1: International Society of Lymphology, Stages of Lymphoedema

	Key features	Recommended therapy	Prognosis
Stage1	Accumulation of protein rich fluid in the epifascial compartment Swelling reduces with elevation or overnight Transient pitting* may occur with moderate pressure Skin appears and feels normal	Therapist oriented treatment for volume reduction Low pressure elastic garments during the day as needed, exercise and skin-care Ongoing self-treatment	With treatment and self-maintenance; No further progression Prevention of acute infections (cellulitis) May revert to stage 0
Stage2	Persistent swelling not relieved by elevation Pitting persistent for minutes or hours Accumulation of adipose tissue and fibroplasia of the epifascial compartment	Decongestive therapy in two phases; Phase 1: Therapist massage, multiplayer compression bandaging, exercise and skin care Phase 2: Daily self-massage, elastic garments, exercise and skin-care.	With comprehensive 2 phase treatment; Reduction of limb volume and softening of fibrotic induration Prevention of acute infections (cellulitis) No further progression Stage 2b may revert to stage 2a
Stage3	Excess subcutaneous fat and fibrosis have replaced the excess free fluid. Only shallow pits can be achieved with firm pressure and may be complete absent. Trophic skin changes may be present such as; hyperkeratosis, persistent skin folds, papillomatosis, lymphoceles, ulcerations and warty overgrowths	Decongestive therapy in two phases as for Stage 2 with the possible addition of; Prophylactic antibiotic therapy Specialise lymphoedema appropriate liposuction Debulking surgery	With comprehensive 2 phase treatment; Reduction of limb volume and softening of fibrotic induration Improvement in skin pathologies Prevention of acute infections (cellulitis) No further progression May revert to stage 2a

** Pitting is the appearance of a skin indentation created by firm thumb pressure maintained for up to one minute which remains visible after the pressure is released.*

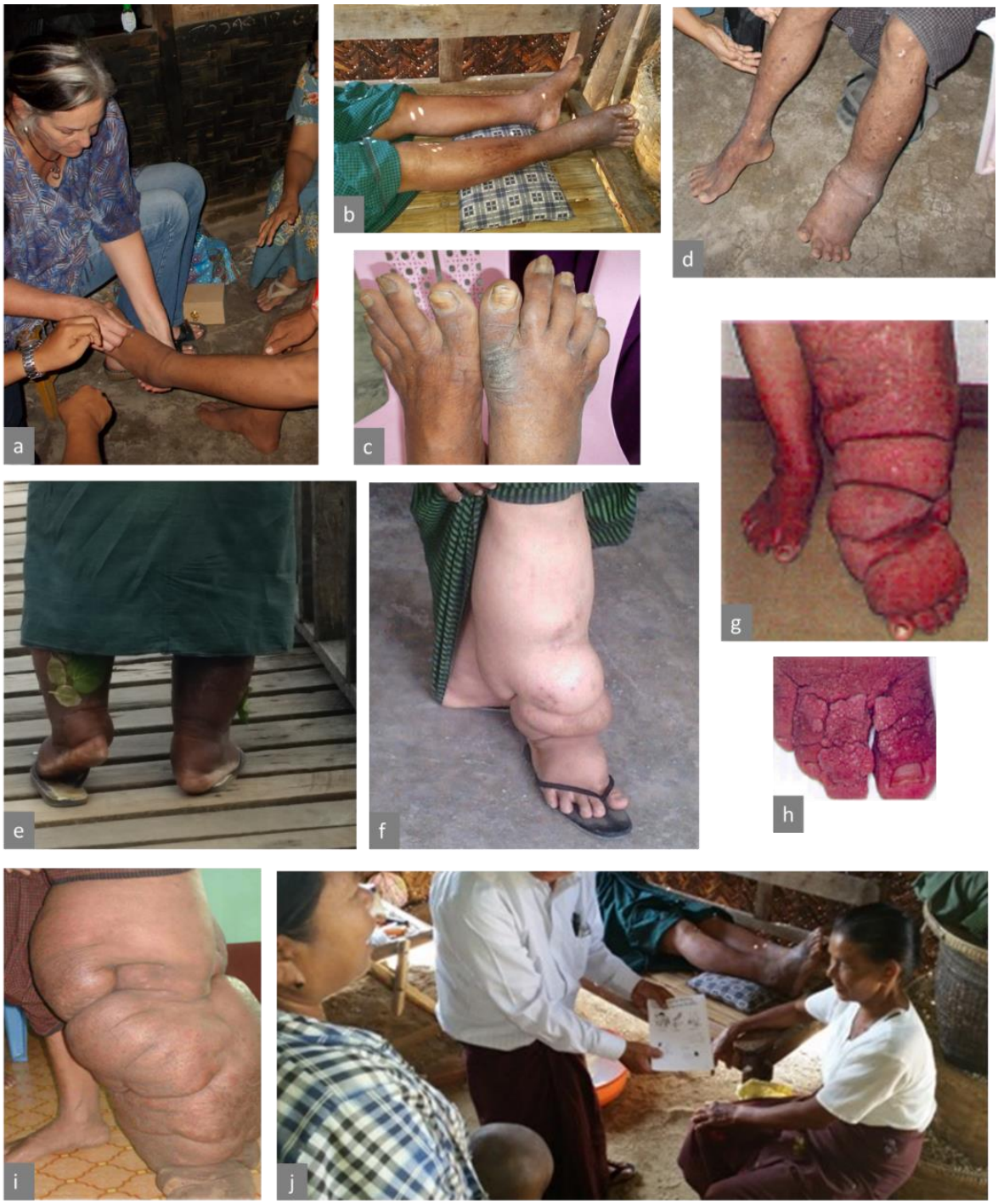
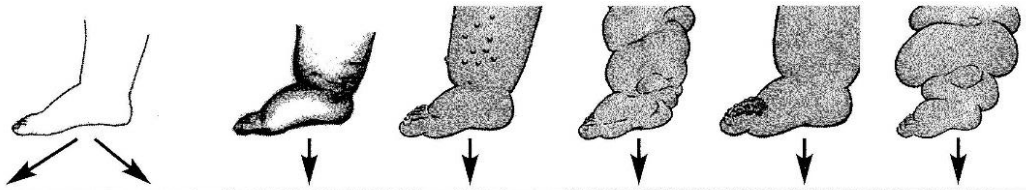


Plate 2.3.1.2: Seven stage criteria as described by Dreyer et al (2002) (description on next page)

Plate 2.3.1.2: (previous page) Seven stage criteria as described by Dreyer et al (2002) a) Assessing filariasis-related lymphoedema (FRL) in Magwe Region Myanmar, Stage 1 swelling goes away on bed rest or overnight, b) Stage 2 swelling does not reduce with elevation, there may be acute attacks and entry lesions especially between the toes, c) Stage 2 FRL of the forefoot and toes, d) Stage 3, shallow skin folds, this young man had regular acute attacks every 4 – 6 weeks, e) Stage 4 presence of knobs or other skin lesions, this person is using a traditional Myanmar remedy of applying leaves to open wounds, f) Stage 5, deep skin folds, g) & h) Stage 6, mossy lesions (papillomatosis), i) Stage 7 gross swelling, frequent acute attacks and inability to perform routine daily tasks. There will be entry lesions between the toes and if basic hygiene is not performed frequently a strong bad odour, j) delivering education in basic hygiene and foot-care to a family in Myanmar. Photographs a), b), c), d), e), f) & j) taken by Jan Douglass. Photographs g) & h) from 'Basic Lymphedema Management, Treatment and Prevention of Problems Associated with Lymphatic Filariasis' Dreyer, Addiss, Dreyer, & Noroes, 2002. USA: Hollis Publishing Company.

LYMPHOEDEMA MANAGEMENT, BY STAGE



Treatment Component	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7
Hygiene (washing and drying)	Yes (ideally at night)	Yes (ideally at night)	Yes (ideally at night)	Yes (ideally at night)	Yes (twice a day if possible)	Yes (twice a day if possible)	Yes (twice a day if possible)
Care of entry lesions	If present	If present	If present	If present	If present	If present	If present
Exercise	Yes	Yes	Yes	Yes	If possible	If possible	If possible
Elevation	Usually not necessary	At night	Day and night	Day and night	Day and night	Day and night if possible	Day and night if possible
Prophylactic creams	No	No	Usually not necessary	Usually not necessary	Usually necessary	Necessary	Necessary
Prophylatic systemic antibiotics (send to doctor)	No	No	No	Usually not necessary	Usually necessary (if acute attacks persist)	Necessary	Necessary
Cosmetic surgery	Not applicable	Not applicable	Not applicable	If medically indicated	If medically indicated	If medically indicated	If medically indicated

Plate 2.3.1.3: Recommended treatment for the seven stages of filariasis-related lymphoedema (Dreyer et al., 2002)

2.3.2 Latent lymphoedema

There is long-standing and unanimous agreement that the earlier lymphoedema is diagnosed and treated, the better the long term prognosis (Ochsner, Longacre, & Murray, 1940) and that treatment should preferably begin before overt clinical signs can be seen (McElrath & Runowicz, 2000; Stout, Binkley, et al., 2012). A latent or Stage 0 lymphoedema is suggested (Stage 1a) in the most recent ISL consensus document (ISL, 2016) but definitive diagnostic criteria are not clear. The first study to prospectively follow breast cancer patients was conducted by Nicole Stout-Gergich (2008) and used bio-impedance spectroscopy (BIS) to measure free fluid in patients' arms pre- and post-operatively. If a 3% change in BIS values was detected a preventative intervention (light compression sleeve) was applied. This was shown to prevent lymphoedema from forming and this paper has since been cited over 130 times. Self-reported symptoms have also been accepted as criteria for diagnosis of BCRL (Ahmed, Prizment, Lazovich, Schmitz, & Folsom, 2008; Czerniec et al., 2010; Hayes, Janda, Cornish, Battistutta, & Newman, 2008; Norman, Miller, Erikson, Norman, & McCorkle, 2001) and this method has potential as an inexpensive means to identify latent lymphoedema.

Interventional studies on FRL may refer to a Stage 0 when participants with mild or early stage lymphoedema are included (Addiss et al., 2010; Kerketta et al., 2005), but the staging criteria are poorly defined and not universal consistent (Ryan, 2004). Without formal recognition or means to identify Stage 0 in LF endemic populations the opportunity to prevent lymphoedema from forming may be missed. Reliable, low cost devices to provide objective assessment of FRL are needed, in particular a protocol to identify Stage 0 FRL which can be used to trigger and prioritise preventative intervention is missing in GPELF guidelines (Douglass et al., 2016) .

Annual MDA of anti-filarial chemotherapy can prevent future transmission of LF and improvements in surgical management of cancer should reduce the incidence of new BCRL cases, but in both aetiologies onset of chronic symptoms may be delayed for months, years or even decades after exposure to the risk, and people with any impairment to lymphatic function bear a lifelong risk of developing secondary lymphoedema (Lawenda et al., 2009; Nutman, 2013).

2.3.3 Objective measures of lymphoedema status

The diagnosis and assessment of lymphoedema status are problematic and there are no universally accepted criteria. A change in between-arm circumference of more than 2 cm change or an increase in between-limb volume of more than 200 ml are often cited in BCRL (Armer, 2005; ISL, 2016; Lawenda et al., 2009). These criteria may lead to false negative diagnoses in large women or those with a high body mass index (BMI) and to false positives in thinner, smaller framed women (Hayes et al., 2008). Even less uniform criteria are applied in assessment of lower-limb lymphoedema (Leung, Tirlapur, & Meads, 2015). The systemic review on self-care in secondary lymphoedema (Douglass et al., 2016) included 18 studies on FRL and 10 studies on cancer-related lymphoedema (CRL) which used a range of measures. The following sections 2.3.3.1 and 2.4.1 are (edited) excerpts from that review.

2.3.3.1 Results on measures of lymphoedema status - from Douglass et al. (2016).

The available resources in each study setting were reflected in the simplicity or complexity of devices used to measure change in limb status. All studies on CRL used either: water displacement (immersion of the limb into a container of water to determine limb volume), bio-impedance spectroscopy to quantify free-fluid (described further in section 2.3.3.3), perometry (an opto-electric device which calculates limb volume from circumference measures at 3 mm intervals), or a truncated cone formula (limb volume calculated from tape circumference measures at 4 cm intervals). These methods can detect very small changes which, although statistically significant, might be of minimal clinical significance.

In contrast, most FRL studies relied on less precise measures and fewer than one quarter of included studies used either water displacement or a truncated cone formula. A further 18% of studies used three or four fixed circumference points to compare affected and unaffected limbs, or summed, or averaged these measures. These methods, especially summed or averaged circumferences, lack the precision to detect small changes in limb volume. FRL studies frequently relied on assessment by stage and criteria with only three or four groups was common, since even studies which used the seven stage criteria often grouped them into early, moderate or late stage disease. These graduations may lack the precision to detect small changes and participants who changed from a higher to a lower stage or vice versa may not have always been detected, thereby under- or over- estimating the effectiveness of the intervention. Overall studies which used more precise measuring protocols more often reported significant evidence for volume reduction in both settings. The results of the self-care interventions are reported in section 2.4.1.

2.3.3.2 Assessment of free fluid

Homeostasis will preserve the intracellular compartment from fluctuations in fluid levels to prevent cell lysis or crenation (Guyton et al., 1966). Although ICF may be compromised in late stage disease, in the early stage when covert tissue fluid is silently accumulating, only the ECF volume will change (Amann-Vesti, 2008; Jensen, Simonsen, Karlsmark, & Bülow, 2010; Ji, 2008; Wiig & Swartz, 2012). Body composition analysis is a well-developed technology with many households now containing bathroom scales capable of differentiating between muscle and fat mass. Of interest to lymphologists is the capacity of BIS to differentiate segmental fluid loads (Brodie, Moscrip, & Hutcheon, 1998; Lukaski, 2013; York et al., 2009).

2.3.3.3 Bio-impedance spectroscopy

The first report on multi-frequency bio-impedance spectroscopy in assessment of BCRL was in 1996 (Cornish, Bunce, Ward, Jones, & Thomas) and has since become a well-accepted and important tool in both clinical practice and research on BCRL (Cornish et al., 1996; Cornish et al., 2001; Libanore et al., 2011; Ridner et al., 2014). Once the self-adhesive electrodes are attached to the skin, the measures are subject to very little operator influence (Moseley & Piller, 2008; Seo & Choi, 2014). An imperceptible low-voltage, multi-frequency current is passed through the tissues and the resistance (impedance) in discrete tissue components is recorded. Of interest in lymphoedema is the resistance in the extracellular (ECF) fluid compartment. Several devices are in regular use in both clinical and research settings and the SBF7 (Impedimed, Australia) showed excellent reliability in the lower limbs of young Australian and Myanmar cohorts (Douglass, Graves, et al., 2017a) which is in keeping with multiple previous studies (Czerniec, Ward, & Kilbreath, 2015; Dittmar, 2003; Jain, Danoff, & Paul, 2010; Matthie, 2008; Moseley & Piller, 2008). A more detailed description of the SBF7 is given in Chapter 3 and images of the device in use can be seen in Plate 2.3.3.3.

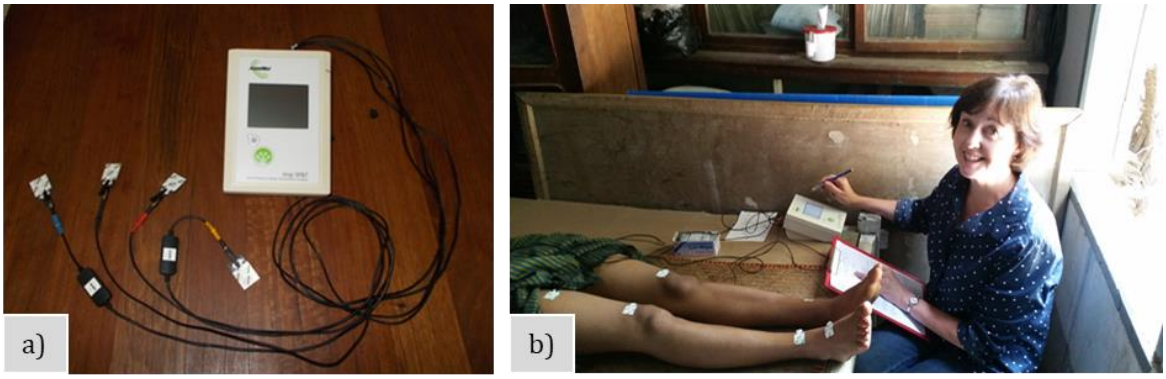


Plate 2.3.3.3: a) The SBF7 unit (Impedimed, Australia) with electrodes attached the electrical leads, and b) Professor Sue Gordon taking BIS measures in Amarapura during February 2015. Photographs taken by Jan Douglass and b) is reproduced with the permission of Sue Gordon.

2.3.3.4 Tissue tonometry (induration)

As lymphoedema progresses, the compressibility or stiffness of the skin and subcutaneous tissue are altered by the presence of fluid, fibre or fat below the skin and as a result of hyperkeratosis of the skin (Szuba & Rockson, 1997). Tissue tonometry (indurometry) can be used to quantify these changes in the skin and subcutaneous tissue. The term 'tonometry' traditionally refers to measurement of intra-ocular pressure (Broman, Congdon, Bandeen-Roche, & Quigley, 2007) and a mechanical hand-held Tonometer (SA Biomedical Engineering, Australia) to measure tonicity in the skin and sub-cutaneous tissue was first described by Clodius, Deak, and Piller (1976). Proposed as an objective measure of fibroplasia in filarial lymphoedema in 1992 (Kar, Kar, & Mania), the main use of the Tonometer has been in assessment of breast cancer-related lymphoedema (BCRL) but several operational issues meant that it was more frequently used in research than clinical practice (Bagheri et al., 2005) and is now no longer manufactured. The device utilised a 200 g mass atop a small indenter extending through a reference plate. When the reference plate is rested flat to the skin the 200 g mass drives the indenter into the skin and underlying tissue and the displacement between the indenter and the reference plate is displayed on an analogue scale. In the early fluid rich stage of lymphoedema, when the subcutaneous compartment is flooded with a gelatinous, high protein oedema, the skin and underlying tissue will be very soft (more compliant) and the indenter will be pressed further into the skin. As lymphoedema progresses fibrosis of the underlying tissue and hyperkeratosis in the skin will make the skin stiffer and the indenter will not be allowed to press as deeply into the tissue.

The original mechanical device (Pallotta et al., 2011) was unable to account for the viscoelastic properties of oedematous tissue. In very soft oedema the indenter may continue to move slowly into the tissue for several minutes which means the analogue dial does not

come to a definitive stop. The operator must then decide at what point to capture the measure and a strict timing protocol is required if repeated measures are used to track changes over time. Also, as the load is generated by the effect of gravity on the 200 g mass, the device must be held within a narrow range (7^0) to vertical in order to maintain the correct load on the indenter and this is difficult to achieve on some body parts.

Evolution of an electro-mechanical device, the Indurometer (SA Biomedical Engineering, Australia), has overcome both these problems by using an in-built force sensor (Vanderstelt et al., 2015). The reference plate is still rested flat to the skin, but the force is applied by the operator pushing the device evenly into the tissue. When the equivalent 200 g force has been applied the device emits a beep and captures the measure in the following microseconds. This timing of the measure at a fixed (almost immediate) time point after the correct load has been applied does not measure the degree of viscoelasticity in the tissue but rather accounts for it by taking all measures within the same time frame. As the device does not need to be held vertically it can be used over body parts at any angle.

Other devices to quantify tissue stiffness or compliance are now available including clinic-based devices which can track complex visco-elastic properties of the skin and underlying tissue (add ref to Clancy 2010). The SkinFibroMeter (Delfin Technologies, Finland) which is a field-friendly handheld device which uses a force sensor to measure resistance to the equivalent of a 50 g mass was also used in this study. More detailed information on the mechanical Tonometer, the Indurometer and the SkinFibroMeter are given in Chapter 3 and images of the devices in use can be seen in Plate 2.3.3.4.

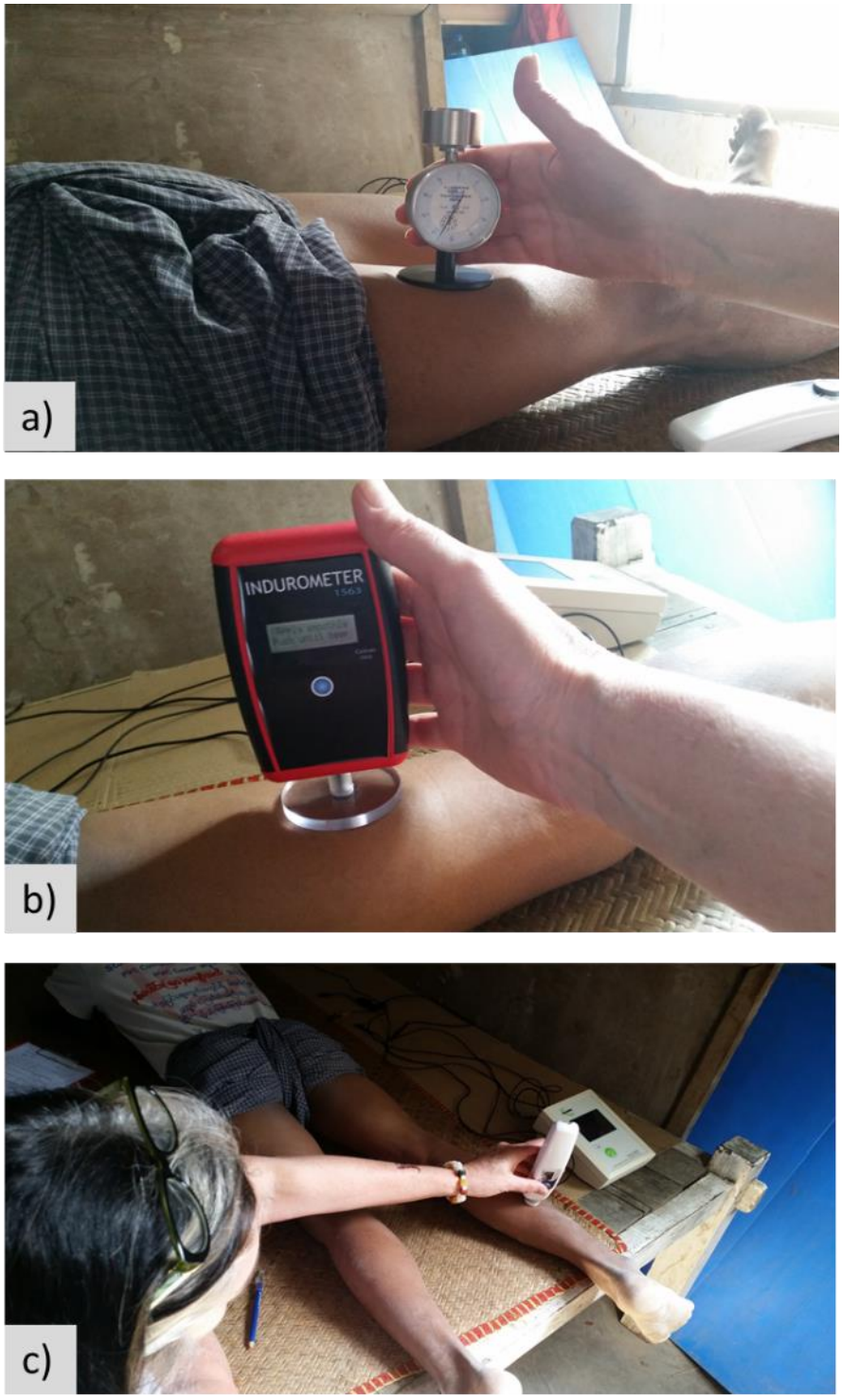


Plate 2.3.3.4: The three tissue tonometers in use in Amarapura during February 2015; a) mechanical Tonometer, Flinders Biomedical Engineering, Australia), b) electro-mechanical Indurometer (SA Biomedical Engineering, Australia, and c) SkinFibroMeter (Delfin Technologies, Finland)

2.3.4 Moderating factors in device measures

Few moderating factors are routinely accounted for in assessment of BCRL other than BMI and arm dominance. Variation in muscle and fat mass are known to influence limb volume, free fluid and lymphatic function (Bourgeois, Leduc, Belgrado, & Leduc, 2010; Czerniec et al., 2015; Dylke, Yee, Ward, Foroughi, & Kilbreath, 2012) and it is likely that these variations will also influence tissue compressibility, but no corresponding data was available prior to publication of the study on young people in Australia and Myanmar (Douglass, Graves, et al., 2017b). Heat is known to increase symptoms in BCRL (Czerniec, Ward, & Kilbreath, 2016; Gordon, Sheppard, & Selby, 2009; Showalter et al., 2013) and increase the rate of normal lymphatic pumping (Groenlund, Telinius, Skov, & Hjortdal, 2017) but since BCRL is typically assessed within airconditioned offices, to determine within-subject tissue changes in post-menopausal women, gender, age, systemic hydration, and menstrual cycle are not considered. These factors could affect BIS and tonometry measures in FRL but prior to the analysis of these devices (Chapter 4) none of these potential moderating factors had been investigated in the lower limb or in young people.

2.4 Lymphoedema management

> *… the most common response that patients received was "do not worry about it, it will go away," or "you will just have to learn to live with it." Of course, once the patient continued on without treatment or education, multiple physical and psychological complications ensued - Saskia Thiadens RN (1998)*

Long considered an intractable non-life-threatening disease, lymphoedema was historically ignored or hidden until disability or infection forced the sufferer to seek medical attention. Even then, the perception that little could be done led to drastic recommendations such as surgical removal of the affected tissue (Evoy & de Takats, 1950). Compounded by a lack of consensus for objective measures, established treatment protocols in all settings were aimed at management of manifest disease and reduction of overt symptoms, rather than prevention of onset (Mitchell, 1995).

Lymphoedema management, as we might now recognise it, was first described in 1892 (von Winiwarter). Although a surgeon, von Winiwarter advocated a conservative approach of meticulous skin-care, limb elevation, massage, compressive bandaging and remedial exercises. In the latter part of the 20th century, primarily as a result of greater survival of cancer patients who frequently developed lymphoedema, these core aspects of treatments were further developed by Vodder, Földi, Asdonk, and Casely-Smith into what is now termed Complex Decongestive (Physio)Therapy (CDT) and which is considered the 'gold standard' in lymphoedema management (E. Földi, Földi, & Weissleder, 1985; A. Williams, 2010).

Ultimately the responsibility for lymphoedema management will fall to the individual and willing family members and one successful program in Kerala, India, which includes both Western and Ayurvedic medicine, requires the inclusion of family members in training and education sessions (Narahari, Aggithaya, Prasanna, & Bose, 2010; Ryan & Narahari, 2012).

2.4.1 Self-care - from Douglass et al. (2016)

As a lifelong condition, true lymphoedema management relies on good self-care, often with the assistance of a carer or partner (Aggithaya et al., 2013; Cimprich et al., 2005; Hagström, 2005; Miller, 1998; Radina, Armer, & Stewart, 2014; Todd, 2014). In FRL the need for self-care is amplified by the lack of specialty trained therapists or access to basic health care of any kind in remote and rural settings. Like CDT, self-management of FRL is based on meticulous skin care, elevation and exercise but the WHO guidelines for these may not be adequate to halt progression or reduce limb volume (Das, Harichandrakumar, Vijayalakshmi, & De Britto, 2013; Wilson et al., 2004). Approaches to self-management in any setting will ultimately be shaped by access to resources and cultural, financial and political influences (Stout, Brantus, et al., 2012) and effective core strategies that can be applied across cultural and economic borders need to be identified. Therefore, a systematic review was undertaken to evaluate the outcome of current self-care interventions for secondary lymphoedema, both cancer-related (CRL) and FRL. Differences and similarities were assessed with respect to self-care components and the results, published in PLOS NTD (2016), have illuminated beneficial practices, that may inform health systems in any setting, to increase the effectiveness of self-care strategies for people with lymphoedema.

Only the main results of the self-care interventions for early lymphoedema are reported here (Section 2.4.1.1). Table 2.4.1 details the contribution of all authors and a full text version of the published paper and supplementary material can be found in Appendix F1.

Table 2.4.1: Contribution of authors to 'Self-Care for Management of Secondary Lymphedema: A Systematic Review.

Author	Nature and extent of contribution
Douglass J.	Developed the search keywords, inclusion and exclusion criteria. Performed the data base searches Previewed search results and excluded articles which did not meet inclusion criteria. Appraised included studies Extracted relevant data from included studies. Performed meta-analysis. Wrote the manuscript and developed figures and tables.
Graves P.	Advised on search strategies. Appraised included studies Provided editorial input for the final manuscript.
Gordon S.	Advised on met-analysis strategies. Provided editorial input for the final manuscript.

2.4.1.1 Abstract (abridged)

Searches were conducted in Medline, CINAHL and Scopus databases in October 2013 and updated in March 2015. Included studies reported before and after measures of lymphoedema status or frequency of acute infections. The methodological quality was assessed using the appropriate Critical Appraisal Skills Program checklist. Descriptive synthesis and meta-analysis were used to evaluate effectiveness of the outcomes reported. Twenty-eight papers were included; two RCTs were found to have strong methodology, and overall 57% of studies were rated as methodologically weak. Evidence from filariasis-related lymphoedema studies indicated that hygiene-centred self-care reduced the frequency and duration of acute episodes by 54%, and in cancer-related lymphoedema home-based exercise including deep breathing delivered significant volume reductions over standard self-care

alone. Intensity of training in self-care practices and frequency of monitoring improved outcomes. Cultural and economic factors and access to health care services influenced the type of intervention delivered and how outcomes were measured.

2.4.1.2 Effects of Basic self-care for FRL

Seven studies (n = 1073) reported on change in either objective assessment of lymphoedema stage or limb volume, or participant perception of limb status. WHO staging criteria was used more frequently (four studies) than the seven stage Dreyer system (two studies). Limb volume was quantified by water displacement (three studies) or limb circumference (three studies).

In a study which excluded participants with stages 5-7 using the Dreyer et al. (2002) criteria, limb volume reduced in stages 0-4 after 12 months and this was significant in stages 2 and 3 which reduced by 4% and 17.1% respectively (Addiss et al., 2010) (Table 2.4.1.2.1). In a cohort with unilateral leg lymphoedema, participants who reported a perceived reduction or no change in limb volume (54%) were more likely to have earlier stage FRL whereas participants who reported a perceived increase (46%) were more likely to have later stages of disease (Das et al., 2013).

Table 2.4.1.2.1: Change in FRL limb volume after 12 months of self-care in Addiss et al (2010)

Stage #	Baseline Mean ml (range) *	After 12 months Mean ml (range) *	N (legs)	% reduction	P
0	1610 (1080-2160)	1604 (1310-2130)	26	0.3%	
1	1937 (1515-2760)	1786 (1470-2030)	9	7.8%	
2	1986 (1450-2835)	1909 (1230-2970)	41	4.0%	p<0.05
3	2839 (1920-3700)	2354 (1580-3260)	15	17.1%	p<0.05
4	3644 (2390-4760)	3082 (1510-4010)	5	15.4%	

*# Dreyer et al. (2002) ml = millilitres * Water displacement method*

Three reports on two studies (n=533) found a significant proportion of participants had reverted to a lower stage after 12 (Wijesinghe, Wickremasinghe, Ekanayake, & Perera, 2007) and 24 months (Budge et al., 2013; Mues et al., 2014) of basic self-care. This effect was greater in participants with early or moderate stage FRL, whereas the proportion of people with more advanced disease either stayed the same (Mues et al., 2014) or increased slightly (not significant) (Table 2.4.1.2.2). Other studies reported no change in lymphoedema stage (Mand et al., 2012; Wilson et al., 2004).

Table 2.4.1.2.2: Proportion of Participants by FRL stage after 6 - 24 months of self-care

Study ID	Stage	Baseline	6 months	12 months	24 months
Mues et al (2014) and Budge et al (2013)[1]	1 - 2	48.73%	54.01%	55.76%	60.13% p=0.0064
	3 - 4	37.57%	32.41%	30.22%	25.32% p=0.0006
	5 - 6	12.70%	13.58%	14.02%	14.56%
Wijesinghe et al (2007)[2]	I	8.6%	n/a	16%*	n/a
	II	52.7%	n/a	46%	n/a
	III	31.3%	n/a	31%	n/a
	IV	7.4%	n/a	8%	n/a

1 = Stages per Dreyer et al (2002)
2 = Stages per WHO 2003 (WHO, 2003)
**Eleven people reverted from Stage II to Stage I (p=0.012)*

2.4.1.3 Basic Self-Care for CRL

No study assessed the effect of self-care alone on CRL.

2.4.1.4 Self-care using topical medication for FRL

Five studies (n= 460) used medicated creams or soap in the daily self-care protocol. Three RCTs were used to compare either medicated soap (Addiss et al., 2011) or medicated cream (Joseph et al., 2004; Shenoy, Kumaraswami, Suma, Rajan, & Radhakuttyamma, 1999) to plain

soap or plain cream, and medicated ointment was used daily by one self-care group in two other trials (Kerketta et al., 2005; Shenoy et al., 1998). All interventions were of 12 months duration and three studies followed participants for a further year after the intervention ceased (Joseph et al., 2004; Shenoy et al., 1999; Shenoy et al., 1998).

In a 12-month intervention on unilateral leg lymphoedema (Kerketta et al., 2005), raw circumference values for the affected limb reduced between 27.6% - 92% with the greatest reduction at the calf of participants with Grade 2 FRL (WHO grades 1 - 3 (WHO, 1985)) and the least reduction at the ankle in participants with Grade 3 (Figure 2.4.1.4). In this trial the difference in circumference between affected and unaffected limbs also reduced significantly at all time points.

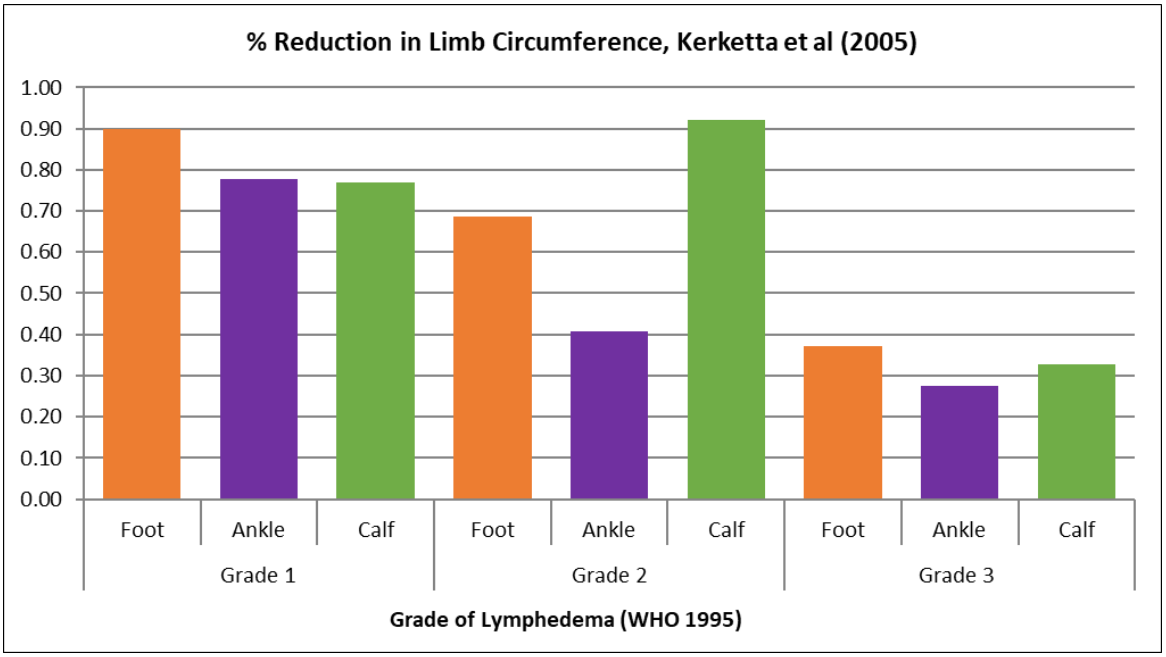


Figure 2.4.1.4: Change in FRL limb circumference after 12 months of medicated cream use

2.4.1.5 Home based exercise for FRL

No studies assessed the effect of prescribed exercises on FRL.

2.4.1.6 Home based exercise for CRL

Seven studies assessed exercise interventions of between eight weeks and six months duration. All participants were women with unilateral arm lymphoedema after breast cancer (n=197) and six studies reported significant benefits in at least one outcome but no CRL studies reported on outcomes by lymphoedema stage.

Results of three studies that reported on change in relative limb volume (RLV) (difference between the affected and unaffected limbs) were combined to estimate the effect of home exercise (Figure 2.4.1.6, n = 54). A random effects model was used due to a high level of heterogeneity between results (I^2 =0). Overall, RLV reduced by 1.31% (95% CI -4.73, 2.11).

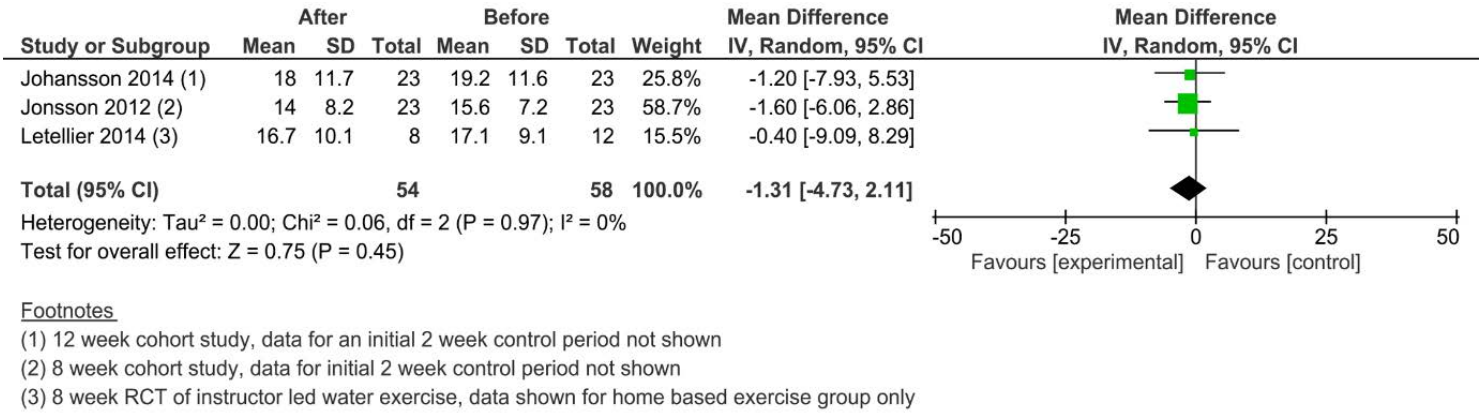


Figure 2.4.1.6: Forest plot of percentage change in relative CRL limb volume after exercise.

Statistically significant reduction in limb volume was recorded 10 minutes after commencing a deep breathing exercise with gentle arm movements (Moseley, Piller, & Carati, 2005), after eight weeks of pole walking (Jonsson & Johansson, 2014) or isotonic arm exercises with deep breathing (Gautam, Maiya, & Vidyasagar, 2011), after ten weeks of weight lifting (Johansson, Klernas, Weibull, & Mattsson, 2014) and in both groups after 12 weeks of either gravity

resisted exercise or self-care with hand pumping (Jeffs & Wiseman, 2013). In the latter trial both groups had reduced further after six months but this was significant only in the gravity resisted exercise group. This trend was supported by a reduction in arm volume in the home based exercise group in an eight week trial (Letellier, Towers, Shimony, & Tidhar, 2014) and in a group of women who practiced yoga at home for six months after an initial four week intervention (Douglass, Immink, Piller, & Ullah, 2012) whereas the participants who discontinued yoga practice had experienced an increase in arm volume (not significant).

2.4.1.7 Summary discussion on the systemic review of self-care

Marked differences were apparent both between and within settings and key opportunities for improvements were identified. Evidence from the FRL population showed that basic self-care alone is effective in preventing ADLA which is consistent with a similar review of the effect of hygiene-based interventions on FRL (Stocks et al., 2015). Hygiene alone may halt disease progression in FRL but is less likely to reduce limb volume. Reduction in limb volume was more difficult to achieve in later stages regardless of the setting and hygiene-centred, basic self-care reduced FRL limb volume only in very early stages. Evidence from the CRL population indicated that greater volume reductions are achieved when activities such as progressive resistance exercise are included in the self-care routine. Since publication of the 2005 study by Moseley et al. (2005), specific deep breathing exercises appear frequently in interventions for CRL (included in 6 studies). Home based exercise, including deep breathing, is easy to perform, requires no financial resources, can be continued alone after minimal initial instruction, and may contribute to overall improvement in health and wellbeing. Simple resistance exercise and deep breathing could be easily incorporated into basic self-care protocols for FRL, particularly in cultures where activities such as Yoga or Tai Chi may be readily available and acceptable.

Whilst basic self-care was the primary intervention in almost 60% of studies on FRL, no CRL studies assessed basic self-care alone; rather the self-care group when included were always as controls. Best practice guidelines in CRL management are still dependent on therapist performed interventions and evidence from the FRL population suggests that more effort to involve CRL patients in their own self-treatment may relieve the financial burden of therapist based care in this population.

This first review to systematically examine the similarities and differences in self-care for CRL and FRL has opened a pathway for further investigation of transferrable strategies for lymphoedema management in disparate settings.

2.5 Economic benefits of proactive preventive interventions

90% of global health care is spent on diseases that affect only 10% of the population – Michael Pollastri, Professor of Medicinal Chemistry

The core basis for addressing NTDs such as lymphatic filariasis is to improve the lives of the poorest of the poor and enable them to lift themselves out of poverty, but surprisingly few economic studies have been performed on the financial benefits of LF elimination (Chu, Hooper, Bradley, McFarland, & Ottesen, 2010; Jordan, 1955; Mattos & Dreyer, 2008; Ramaiah, Das, Michael, & Guyatt, 2000). Even fewer economic studies have determined the direct benefit to communities of delivering MMDP activities in the GPELF. Economic modelling has shown the estimated lifetime per-person saving in medical costs and earnings losses due to chronic FRL to be 130 times the per-person investment in implementing basic limb-care (Stillwaggon et al., 2016). Such a profound improvement in the local economy has yet to be measured directly.

The economic cost of lower limb lymphoedema in CRL is completely unexplored but several studies have attempted to quantify the financial burden to women with BCRL of the arm (Chirikos, 2001; Chirikos, Russell-Jacobs, & Jacobsen, 2002; Schmitz, DiSipio, Gordon, & Hayes, 2015). Whilst it may seem intuitive that early intervention will reduce the lifelong cost of living with lymphoedema, no studies have measured these benefits directly. Modelling by Nicole Stout and colleagues (2012) in the American health system has estimated the annual per-person cost of managing early-stage BCRL compared to late-stage BCRL to be USD 636.19 vs USD 3,124.92 respectively. A better understanding of the indirect costs such as the burden of mental health issues and dependence on family carers, will support the cost-effectiveness of earlier intervention for lymphoedema from all causes. The current lack of direct evidence for the financial benefits to people with lymphoedema and their communities in either setting

contributes to the lack of financial commitment to lymphoedema management in public health policy worldwide (Stout et al., 2013).

2.6 Key points

- Lymphoedema has been identified in recorded history for millennia and is a major cause of disability worldwide.
- Filariasis-related lymphoedema causes most of the burden of lymphoedema globally.
- The global LF elimination program (GPELF) aims to interrupt disease transmission by 2020.
- W. bancrofti is endemic in Central Myanmar but the national LF elimination program in is lagging behind its South-east Asia neighbours.
- When normal lymphatic function is hampered, imperceptible subcutaneous oedema can develop and progress to overt lymphoedema.
- Lymphoedema is a secondary tissue disease which develops as a consequence of persistent insufficiency in lymphatic transport.
- Manifest lymphoedema is irreversible and progression to advances stages is accompanied by increasing deformity and disability.
- Regardless of the aetiology, pathogenesis of lymphoedema usually follows a course of predictable changes in tissue composition
- Onset of chronic symptoms may be delayed for months, years or even decades after exposure to the risk

- Monitoring of covert symptoms and early intervention in persons at-risk of BCRL can prevent lymphoedema forming.
- It is not yet clear if annual MDA of PC will reverse early stage disease in FRL.
- There is no formal recognition of Stage 0 or agreed guidelines to identify at-risk cases.
- Intervention strategies vary widely depending on the setting.
- Evidence for self-care in FRL management indicates that this strategy should be expanded and promoted in all settings.
- No studies have measured the direct economic benefit of early intervention in disease management.
- Investigation of low-cost objective assessment methods for FRL are urgently needed.

Chapter 3: Data collection methods for all studies

A description of the research activities and detailed methods for data collection in all studies are reported in this Chapter. Related research activities can be viewed in Figure 3.

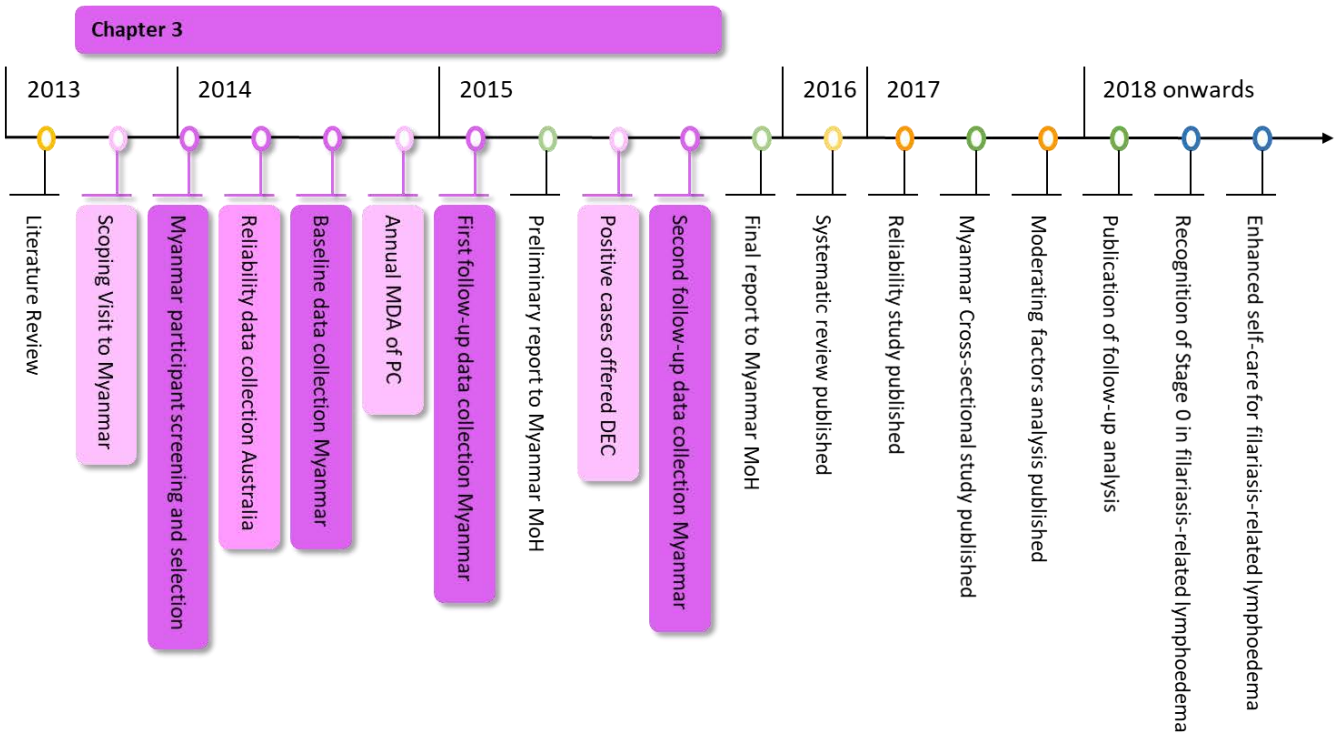


Figure 3: Research activities related to data collection in all studies

3.1 Study sites

The pilot study in PNG that inspired this thesis was conducted in Opau village, Gulf Province, but the distributed population and inaccessibility of LF endemic villages made PNG unsuitable for a longitudinal study. In consultation with Louise Kelly-Hope at the Centre for Neglected Tropical Diseases, Liverpool School of Tropical Medicine (UK), Myanmar was identified as an accessible country with several regions with a high prevalence of LF microfilaremia (Dickson et al., 2017).

3.1.1 Myanmar

Preliminary discussions with the Myanmar National LF Program Manager, Dr Ni Ni Aye, led to permission to travel with a WHO observation team during the 2013 MDA. Health and administration centres were visited in Mandalay and Magwe Regions and sentinel site records held by Vector Borne Disease Control (VBDC) Mandalay office were used to identify suitable study locations. Amarapura Township was selected on the basis of population density, high LF prevalence, and accessibility to laboratory services. A data collection centre was set up in the Administration Centre in the village of Nge Toe in October 2014 with the permission of the administrator and all blood collection and device measures were performed within the centre. The same study centre was used at both follow-ups in February and June 2015 and a photograph of the administration centre, administration centre staff and research assistants can be seen in Plate 3.1.1.

Plate 3.1.1: Administration centre, Nge Toe Village, Amarapura Township, administration staff, research assistants and Jan Douglass. The photograph was taken by an administration centre staff member and later framed and presented to the Administrator.

3.1.2 Australia

Measures on young Australian participants in the reliability study were collected in an office on the JCU Townsville campus which was reserved for this purpose between September 2014 and May 2015. Three volunteers in Cairns were measured in the exercise physiology room at JCU Cairns campus. Sample recruitment materials for the reliability study in Australia can be seen in Appendix D.

3.2 Ethical approvals and informed consent

Ethical approval for the study was provided by the James Cook University (JCU) Human Research Ethics Committee (HREC approval number H5261) and was contingent upon approval from the Myanmar Ministry of Health and Sports (MOHS). During the 2013 visit a meeting with the Director General of the Department of Health was held and two amendments to the original proposal were requested, 1) that the age of Myanmar participants be restricted to children 10 years or older, and 2) that a morbidity survey be conducted. These amendments were made and ethical approval to conduct the study was given in May 2014 by the Myanmar Ministry of Health. All ethical approvals can be viewed in Appendix B.

The morbidity survey was conducted as the final year honours project of Dr Benjamin Dickson and the results were presented at the 64th Annual Meeting of the American Tropical Society of Medicine and Hygiene (Dickson et al., 2015). A further ethical approval for the reliability study was obtained from JCU HREC to include an Australian cohort (approval number H5497, Appendix B3).

In Myanmar, participant information sheets and consent forms were translated by MOHS staff, checked by WHO staff, and provided to participants in Burmese. In Australia all documentation was in English. In both locations, written consent was given by young adults aged 18 – 21 and by a parent or guardian of minors up to 17 years of age. A further verbal assent for each procedure was obtained from all participants prior to performing that procedure and for all minors the parent or guardian was present during data collection procedures. Participant information sheets and copies of the consent forms are provided in Appendix C.

3.3 Devices of interest

The systematic and narrative reviews were used to identify devices which were well validated in research on BCRL and which could be suitable for poorly resourced and remote settings. Tissue tonometry and bio-impedance spectroscopy were chosen as they are small hand-held or desk-top devices which are easily transportable and do not rely on a constant power source. They are inexpensive to procure and require minimal operator training. Whilst they appear frequently in BCRL literature there has been limited application in LF populations or in field conditions (Gordon et al., 2011).

3.3.1 Tissue tonometry

The tissue tonometer used in the pilot study in PNG (Gordon et al., 2011) was a purely mechanical device called the Tonometer (Flinders Biomedical Engineering, Australia). The Tonometer was used in Myanmar but not in Australia. In both locations two electro-mechanical devices were also used: the Indurometer (SA Biomedical Engineering, Australia) and the SkinFibroMeter (Delfin Technologies, Finland). All three instruments are small hand-held units and the digital versions are powered by internal batteries. The Tonometer and the Indurometer were supplied by the JCU School of Physiotherapy and the SkinFibroMeter was supplied on loan from Delfin Technologies, Finland.

3.3.1.1 Mechanical Tonometer (Flinders Biomedical Engineering, Australia)

This device uses gravity to load a 1 cm diameter indenter which extends through an opening in a 7cm diameter reference plate (Plate 3.3.1.1). The reference plate is rested flat on the skin and a 200 g mass drives the indenter into the skin and underlying tissue. Tissue stiffness - the degree of resistance to the indenter - is shown on two analogue displacement gauges in 0.10 mm and 0.01mm increments. Higher values are recorded when the indenter is able to press

deeper into the tissue and are an indication of softer (more compressible) tissue. This device was recalibrated daily by resting the device on a hard, flat surface and adjusting both analogue dials to zero.

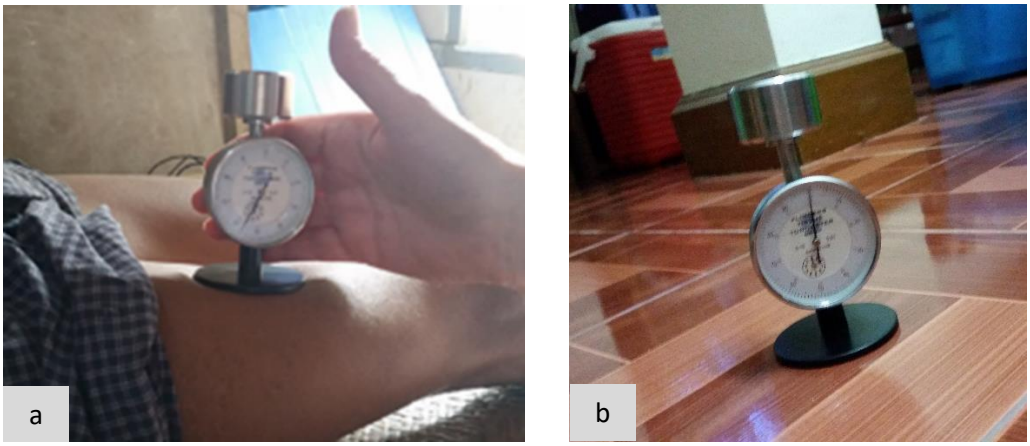


Plate 3.3.1.1: Mechanical Tonometer (SA Biomedical Engineering, Australia) a) in use in Myanmar and b) during daily recalibration

3.3.1.2 Indurometer (SA Biomedical Engineering, Australia)

Developed to overcome the operational issues related to gravity and positioning inherent in the mechanical device, the Indurometer uses a force-sensor to detect when the equivalent to a 200 g load has been applied to the tissue. The dimensions of the reference plate and indenter are the same as the mechanical version (see Plate 3.3.1.2). To record the measure, the reference plate is aligned to the surface of the skin while the device is pressed evenly into the tissue by the operator. A beep is emitted once the correct force has been applied and the measure is recorded. The result is displayed digitally in increments of 0.01 on an LED (light emitting diode) screen and remains visible until the screen is cleared when the device is reset for the next measure, or turned off. As with the mechanical Tonometer, the Indurometer

detects the displacement between the reference plate and the indenter and higher values indicate softer (more compressible) tissue.

The inbuilt force sensor issues an error warning if the application is erratic, too slow, or too fast, in which case the device must be re-set and the measure re-attempted. This device was recalibrated daily using the self-calibrating protocol as described by the manufacturer.

Plate 3.3.1.2: Indurometer (SA Biomedical Engineering, Australia) a) during daily calibration and b) in use in Myanmar

3.3.1.3 SkinFibroMeter (Delfin Technologies, Finland)

With a smaller reference plate (2 cm diameter) and a 1.25 mm fixed indenter, the SkinFibroMetre uses a force-sensor to detect resistance to an equivalent of 50 g. The operator uses a supported arm to briefly apply the reference plate five times in the same location and the digital readout displays the average measure of resistance in Newtons (N) in 0.01 increments (see Plate 3.3.1.3). The screen is cleared when the device is reset for the next measure or turned off. With this device, the indenter does not move in relation to the reference plate and it is the resistance to the indenter which is recorded, therefore higher values indicate more stiffness (less compressibility) in the skin and underlying tissue. According to the manufacturer's instructions this device should be returned for recalibration every two years.

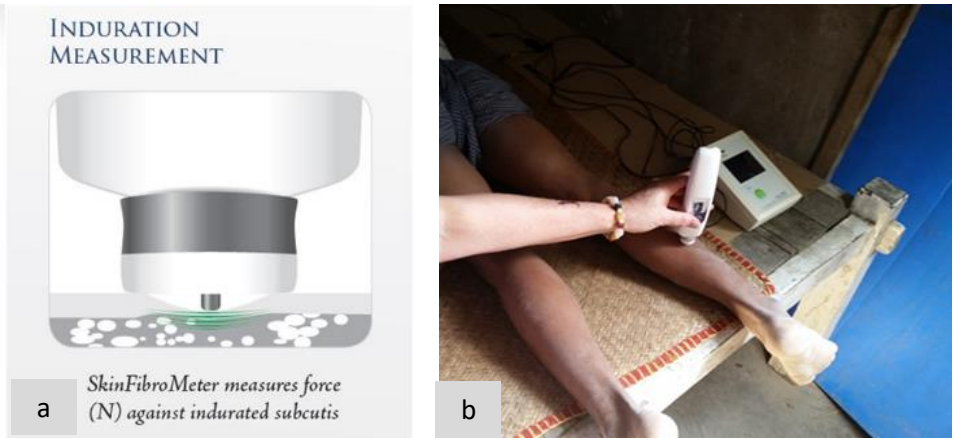


Plate 3.3.1.3: SkinFibroMeter (Delfin Technologies, Finland) a) manufacturer's schematic of the measurement principle and b) in use in Myanmar

3.3.2 Bio-Impedance spectroscopy

Extra cellular and intracellular fluid loads were assessed using bio-impedance spectroscopy (BIS). The resistance in the ICF and ECF were recorded individually and downloaded to an Excel file (Microsoft Office 365, version 1706). A ratio of ICF resistance to ECF resistance was calculated and represented as a ratio (Ri:Re). An increase in free fluid (ECF) will reduce the ratio and therefore lower values represent higher free fluid loads. Previous research has shown that the BIS electrodes were unaffected by increased temperature or sweating (Cornish, Thomas, & Ward, 1998).

3.3.2.1 SBF7 (Impedimed, Australia)

This portable, battery operated multi-frequency analyser uses self-adhesive electrodes to deliver 256 frequencies over the range 3 – 1000 KHz (Plate 3.3.2.1). Low frequencies pass only through the extracellular fluid while higher frequencies pass through both the ECF and the ICF. The electrodes were positioned according to manufacturer's instructions for lower limb measures and the device can store up to 1000 recordings until the data is downloaded. The self-calibrating protocol was performed daily before the first measure.

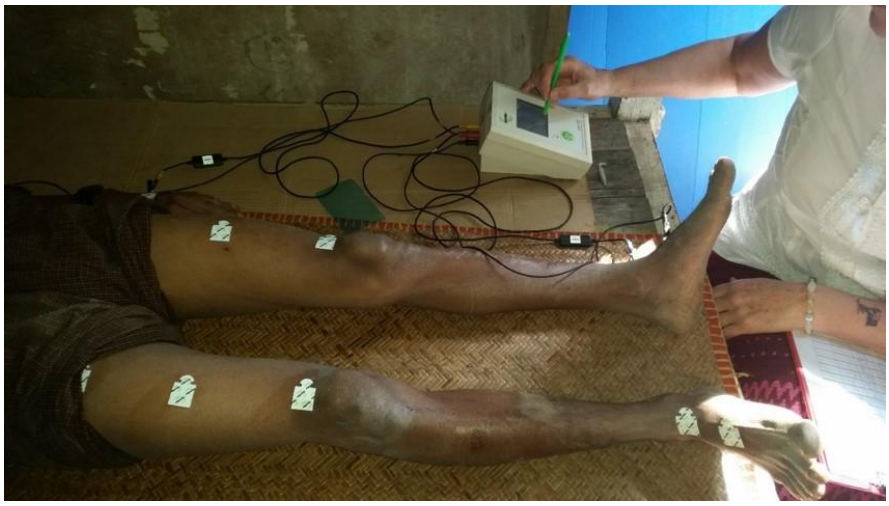


Plate 3.3.2.1: SBF7 (Impedimed Australia) in use in Myanmar

3.4 Participant recruitment and selection

An online calculator was used to estimate a sample size of 32 subjects per group to detect a 10% variation in device measures using an expected mean Indurometer value of 2.5 (SD 0.7) at mid-calf (Douglass, Graves, et al., 2017a) with a 95% confidence interval and 80% power (Holleran & Ramakrishnan, 2013).

3.4.1 Recruitment in Australia

A convenience sample of North Queensland residents aged eight to twenty-one years was recruited through institutional emails and local notice boards (Appendix D1). Electronic and hard copies of the participant information and consent documents were provided in English (Appendix C4 and 5) and the principle researcher (JD) conducted an initial telephone interview to determine eligibility (Appendix D2). Recruitment commenced in September 2014 and continued until May 2015.

3.4.2 Recruitment in Myanmar

Recruitment commenced on October 14th, 2014. Residential block leaders and local administration centre staff invited young people aged ten to twenty-one years to be tested for the presence of LF antigen. Participant information sheets and informed consent forms were provided in Burmese (Appendix C1, C2 and C3). Staff of the VBDC and Amarapura Township Hospital, the WHO LF-technical adviser for Myanmar, and locally trained research assistants, explained all procedures to the participants, witnessed the informed consent forms and determined eligibility to participate.

3.4.3 Inclusion and exclusion criteria

In both locations, participants were excluded if they had any clinical sign of lymphoedema, or any other condition that might affect measures of either leg. Figure 3.4.3 shows the inclusion and exclusion criteria used.

Inclusion criteria • Able to give informed consent (18 – 21 year olds) • Accompanied by an adult relative able to give informed consent (8 – 17 years olds) **Inclusion criteria Myanmar** • Aged 10 – 21 years at screening • Residing in Amarapura Township **Inclusion criteria Australia** • Aged 10 – 21 Years • Residing in North Queensland	**Exclusion criteria** • Any clinical sign of lymphedema • Acute injury to the lower limb(s) • Acute infection of any kind • Past surgery to the trunk or lower limb(s) • Heart disease • Pacemaker • Other implanted device • Pregnancy • Unable to give informed consent

Figure: 3.4.3. Inclusion and exclusion criteria for participants in Myanmar and Australia

3.4.4 Screening for LF infection and participant selection in Myanmar

Volunteers in Myanmar who were eligible to participate in the study were screened using an immuno-chromatographic test (ICT) card (Binax Now, Alere, USA), a rapid field test for the presence of LF antigen. This involves placing a 100 ul draw of blood, collected by heparinised capillary tube from a finger prick, onto the end of the test strip and allowing the sample to flow onto the strip. The sample is read at exactly ten minutes and the result appears as one or two lines across the strip. The far line is a control and if this line is not visible then then not enough of the sample has reached the chromatographic test area and the test is void. Appearance of the second line indicates the presence of circulating *W. bancrofti* antigen from adult worms. Images of recruitment and screening procedures can be viewed in Plate 3.4.4.

Young people who tested positive by ICT (cases) and a sample of negative participants of the same age and gender (controls) were invited to return and participate in the longitudinal study.

Plate 3.4.4: a) A finger prick blood sample collected by heparinised capillary tube, b) an ICT card ready for use, c) ICT cards are marked with the participant ID and the time the test was commenced and read at 10 minutes. The result on this card indicated that the test was valid (the control line is visible) but negative for LF antigen (no test line) and d) the study centre during recruitment and screening activities on 18th October 2015. Photographs a) & d) taken by Kyaw San Tun and reproduce with permission, photographs b) & c) taken by Jan Douglass.

3.5 Data Collection

3.5.1 Participant questionnaires

Prior to physical measures or blood collection, a short interview was conducted to elicit information on current health status, prescription or traditional medications, surgical history, family history of lymphoedema and time since the last drink (as a proxy for hydration). Young women were asked the time (in weeks) since their last menstrual period. Leg dominance was determined by asking the question *Which foot do you use to kick a ball?* and Myanmar participants were asked if they had consumed the preventive chemotherapy drugs during the previous MDA. This questionnaire was repeated at each follow-up and examples of the participant interview forms are provided in Appendix E1.

3.5.2 Physical measures

All measurements were collected between 10am and 3pm. Height in centimetres was measured using a stadiometer in Australia and in Myanmar a chart was marked on a wooden post in centimetres and a set square was used to align the top of the head with the marked scale. In both locations weight in kilograms was recorded using commercially available digital scales. Height and weight data were used to calculate BMI using the formula; kg/m^2. Photographs of height and weight measures in Myanmar can be seen in Plate 3.5.2.

All devices of interest were operated by the principal researcher (JD) who was blinded to the infection status of the Myanmar participants at baseline. In Myanmar, a research assistant who was the same gender as the participant, applied the BIS electrodes and filled in the device scores on the bio-data form. In Australia, the principle researcher applied the BIS electrodes and the parent, a friend, sibling, or the participant themselves, filled in the bio-data form.

Plate 3.5.2: U Win Thu, Health Assistant at Amarapura Township Hospital collects a) height and b) weight measures from a Myanmar participant. Photograph taken by Jan Douglass and reproduced with permission.

For posterior leg measures participants were asked to lie prone on a low bench with their feet hanging over the edge (see Plate 3.5.2.1). For anterior leg measures including BIS measures the participant lay supine with their heels on the bench. Participants were asked to relax throughout the measurement protocols. For each physical measure, the principle researcher stated the measurement location and read the score aloud from the device in use. The scribe then repeated the score aloud and recorded it on the bio-data form. An example of the biodata forms can be seen in Appendix E2.

All devices measures were repeated. A third measure was taken for data sets included in the reliability analysis. Whether two or three measures were taken, the average of all available scores was used in all data analysis. In the Myanmar group, it was originally intended to record triple measures for the reliability analysis at baseline, but the extra measure delayed

the flow of data collection and was abandoned after the first two days. The remaining triple measures on negative participants were collected at first follow-up when data collection methods were more streamlined.

3.5.2.1 Measurement points and leg circumference

A tape measure was used to obtain three segmental lengths on each leg: the calf between the base of the heel and the crease behind the knee, the posterior thigh between the crease behind the knee and the gluteal fold, and the anterior thigh between the superior border of the patella and the crease of the groin. The mid-point of each segment was then calculated by halving the full-length measures and a washable skin marker was used to mark each mid-point on the leg. Circumference measures were taken at the mid-point of the anterior thigh and at the mid-point of the calf. Leg length, mid-point and circumference measures were recorded only once. Plate 3.5.2.1 shows the locations of the mid-points and circumferences measures.

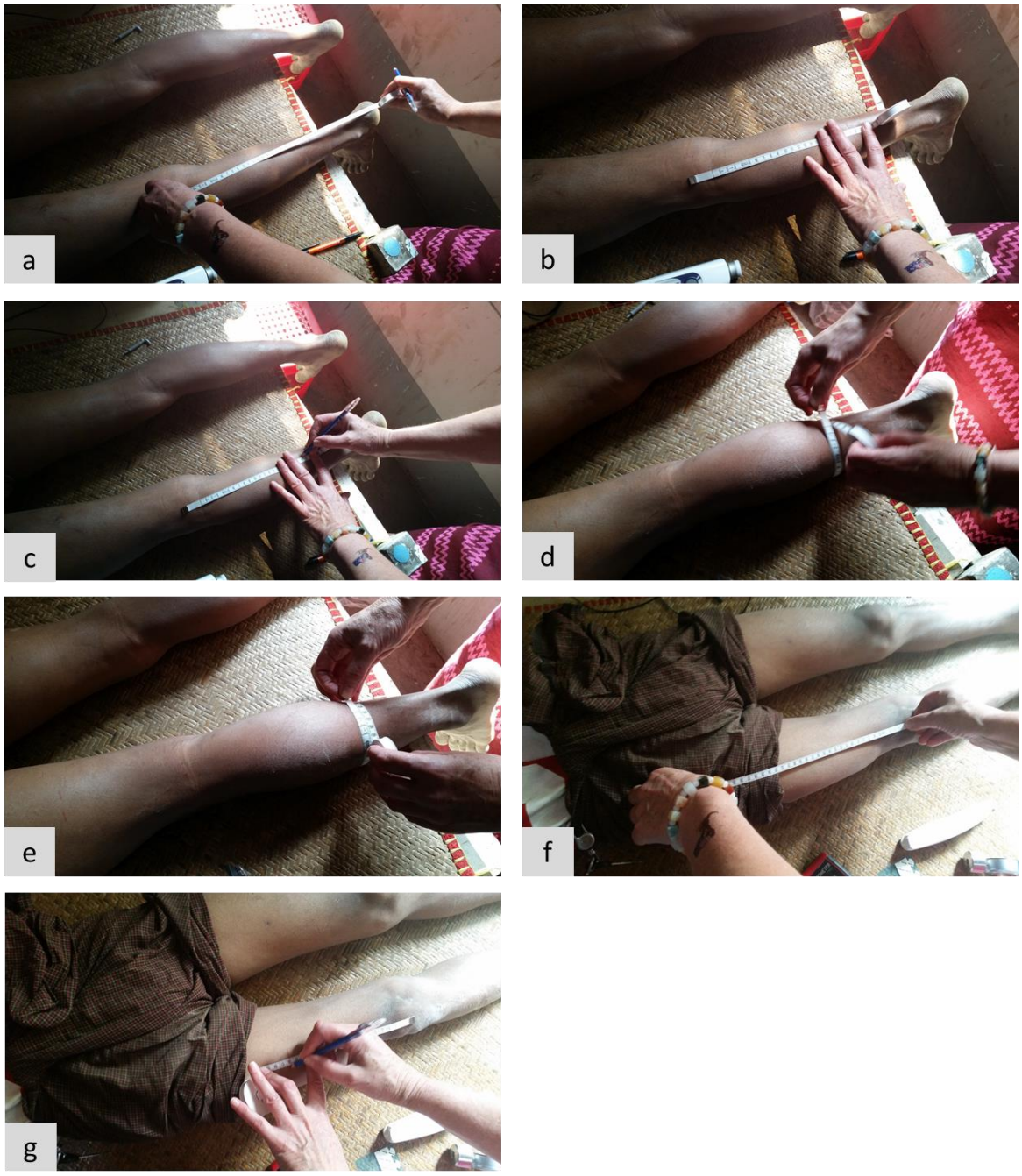


Plate 3.5.2.1: Tape measure to locate the mid-points of the calf and anterior thigh segments and the circumference of the calf. A) measuring the length of the calf segment between the base of the heel and the crease behind the knee, b) stabilising the tape measure at the calf mid-point, c) marking the mid-point on either side of the tape, d) placing the tape around the calf at the mid-point, e) laying the tape measure flat without tension to read the circumference measure, f) measuring the length of the anterior thigh between the superior border of the patella and the crease of the groin, g) stabilising the tape measure around the mid-point of the anterior thigh. Photographs taken by the Myanmar research assistant with verbal permission from the participant.

3.5.2.2 Tonometry measures

All tonometry measures were taken at the marked mid-points of each leg segment. Although persistent indentation should not occur in healthy skin, the devices were used in the following order to avoid any residual pitting from the previous instrument: 1) The SkinFibroMeter which very briefly applies 50 g of pressure: 2) the Indurometer which presses the indenter into the skin with an equivalent to 200 g but is applied only briefly: and 3) the mechanical Tonometer which uses a 200 g mass atop the indenter and must remain on the skin with the force remaining in place while the operator reads both dials. All measures were taken in the order right calf, left calf, right posterior thigh, left posterior thigh, right anterior thigh and left anterior thigh. Blinding to which device was in use was not possible, but following the sequential order of measures and an independent scribe was used to minimise any risk of bias.

3.5.2.3 Bio-impedance measures

The SBF7 was used last after all the circumference and compressibility measures. The skin at the attachment sites were cleaned with an alcohol swab before the self-adhesive electrodes were attached according to the manufacturer's instructions for whole leg analysis. In Myanmar this was done by the research assistant and in Australia by the principal researcher (JD). Both legs were measured, beginning with the right leg, and either two or three measures were recorded depending whether the data was included in the reliability study (3 measures) or not (2 measures). Data was stored on the device until downloaded at the end of each day.

3.5.3 Plasma samples (Myanmar only)

3.5.3.1 Blood collection

A 10 ml draw of venous blood was collected from each participant by a VBDC laboratory technician. Blood was collected into cooled EDTA anticoagulant vacutainers (BD Biosciences, Australia) and 100 ul was immediately pipetted onto an ICT card to repeat the antigen test. A further 60 ul was pipetted in 10 ul portions to six petals of a filter paper and dried. Plate 3.6.3.1 shows a blood sample being collected.

Plate 3.5.3.1: Thiha So, laboratory technician at Mandalay Region Vector Borne Disease Control, collects a 10 ml venous blood sample. 100 ml was used to repeat the ICT test and 60 ml was pipetted onto the petals of a filter paper. The remaining blood was kept on ice until transferred to the Public Health Laboratory in Mandalay for separation. Photograph taken by Jan Douglass and reproduced with permission.

3.5.3.2 Plasma separation

The remaining whole blood was kept on ice until transfer to the Public Health Laboratory (PHL) in Mandalay. Separation of plasma and red blood cells was performed within four hours of collection by PHL laboratory technicians using a centrifuge for 15 minutes at 3000 rpm. The plasma was pipetted into 2 ml cryo-tubes in duplicate (4 ml per person) and samples were stored at the PHL in Mandalay at -20^{0}C until the end of each data collection period. Plate 3.5.3.2 shows the PHL technicians and frozen cryotubes of plasma.

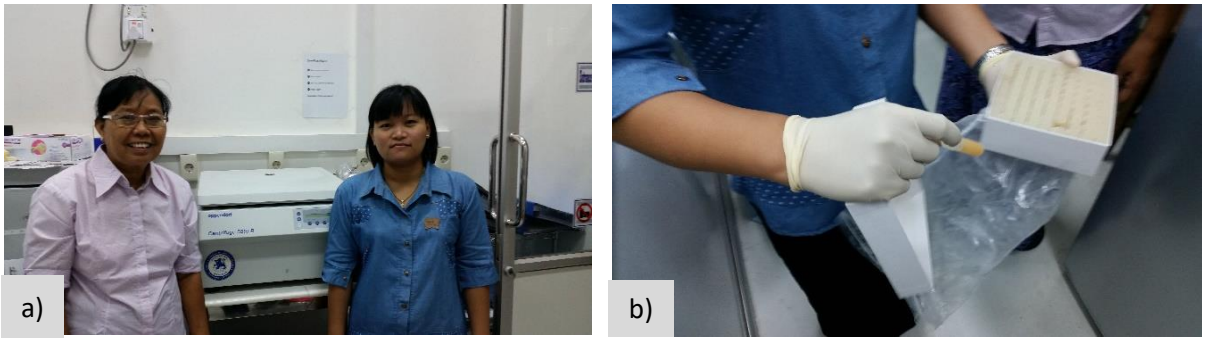


Plate 3.5.3.2: Blood separated at the Public Health Laboratory in Mandalay by Dr Yi Yi (laboratory director) and senior technician, Thi Thi Lwin (a) and b) Thi Thi Lwin confirms the plasma is frozen before transfer on dry ice to Yangon. Photographs taken by Jan Douglass and reproduced with permission.

3.5.3.3 Plasma storage

After each data collection phase, the plasma samples were packed in dry ice and taken by road to Yangon where they were stored at the Department of Medical Research (DMR) in a digitally monitored -80^{0}C freezer connected to a back-up generator. There were no thaws during plasma transportation or storage. Plate 3.5.3.3 shows the samples being transferred to the Department of Medical Research in Yangon.

Plate 3.5.3.3 a) Plasma samples were packed in dry ice and transported by car to b) the Department of Medical Research in Yangon where they were received by Dr Pa Pa and c) stored at -80C. Photographs taken by Jan Douglass and reproduced with permission.

3.5.3.4 Serological analysis

One set of the cryotubes for each person was thawed at DMR and 100 ul was used to confirm the infection status of participants. The remaining portion of the thawed plasma was refrozen at -80^{0}C in preparation for transfer to the Neglected Tropical Diseases Laboratory at JCU, Cairns, Australia.

In Myanmar, Og4C3 monoclonal antibody-based ELISA (enzyme-linked immunosorbent assays) (Cellabs, Australia), which uses monoclonal antibodies to detect antigens produced by adult filarial worms, were used to confirm infection status. The 1:4 dilution as per manufacturer kit instructions was used and samples were classified as positive if the antigen units estimated using the standard curve of controls provided with the kit exceeded 32 units. Plate 3.5.3.4 shows the laboratory staff involved in serological testing in Myanmar. The Og4C3 ELISA assays were repeated in Australia by Jesse Masson as part of his Masters in Public Health. The results of these serological analyses were published in the journal Tropical Medicine and Infectious Diseases in 2017 (Masson et al., 2017a, 2017b).

Plate 3.5.3.4: Maureen Roineau, laboratory technician at the JCU NTD laboratory, JCU Cairns, and laboratory technicians at the Department of Medical Research in Yangon. Photograph taken by Jan Douglass and reproduced with permission

3.5.3.5 International transfers

The second 2 ml cryotube was transferred to Liverpool School of Tropical Medicine Parasitology Laboratory for future analysis of biochemical markers of covert FRL. These results will form a part of the research of several higher degree students and will not be reported in this thesis. Both international transfers were completed by placing the frozen tubes into labelled bio-pouches in an insulated and correctly labelled bio-box (Bio-Bottle Australia, Box type BZ20). The box was packed with commercially available freezer blocks which had been frozen to -80^{0}C, and absorbent paper. Both freezer to freezer transfers were made as excess passenger baggage and completed within 24 hours of removing the samples from the first freezer. After both transfers the cryotubes arrived frozen and were immediately transferred into -80^{0}C storage at the receiving laboratories. Plate 3.5.3.5 shows the frozen cryotubes arriving at the destination laboratories.

Plate 3.5.3.5: Frozen cryotubes arriving still frozen at a) NTD laboratory, JCU Cairns (Luke Becker) and b) Parasitology Laboratory at Liverpool School of Tropical Medicine. Photographs taken by Jan Douglass and reproduced with permission.

3.6 Data Analysis

Four separate analyses were conducted on the physical data. The first two analyses included: Myanmar who had tested negative by ICT for LF-antigen, and all the Australian participants, and focused on establishing device reliability and identifying moderating factors in device scores. Results for these analyses are provided in Chapter 4. The second two analyses on the Myanmar data only, compared antigen positive and negative groups at baseline (Chapter 5) and LF-positive cases before and after preventive chemotherapy (Chapter 6). Analysis for each study was conducted separately and a brief overview of the variables analysed, outcomes assessed, and tests used are given here. Descriptions of study specific analysis are given at the beginning of the relevant results Chapter.

3.6.1 Analysis of intra-rater reliability of device measures

The average of three measures for each device at each measuring point were used in data analysis. An intra-class correlation coefficient (ICC) was used to determine the intra-rater reliability and one-way repeated measure analysis of variance (ANOVA) was used to obtain within subject variance. Since each device returns a different unit of measure a coefficient of variation (COV) was used to determine the agreement between devices at each measuring location. All analysis was performed using STATA 12 (StataCorp USA). Results of the reliability analysis are presented in Chapter 4.2.

3.6.2 Analysis of moderating factors associated with variance in measures

Data from all the Australian participants and from the Myanmar participants who tested negative by ICT at baseline were included in this analysis. A variable to identify underweight participants was calculated using Centre for Disease Control (CDC) charts and criteria accessed online during June 2016 (CDC, 2015). Younger (8 – 16 years) and older (17 – 21

years) group variables were used to assess the influence of normal growth on device scores. Between-country comparisons were made using independent samples T-tests for continuous variables and Chi-Square or Fisher's exact tests for categorical variables. Paired samples T-tests were used for between-leg comparisons (dominant vs non-dominant). Linear regression was used to determine how moderating factors were associated with variance in scores. All analysis was completed using SPSS Version 23 (IBM Corp, USA). Results of the analysis on moderating factors in device scores are presented in Chapter 4.3.

3.6.3 Cross-sectional analysis of baseline data

LF antigen positive cases were defined as those who were positive by either antigen test (ICT or Og4C3 assay). WHO growth charts and definitions for BMI-for-age were accessed online during March 2016 (WHO, 2009) and used to identify underweight participants. Chi-squared tests, Fisher's exact tests and independent sample t-tests were used to compare antigen positive and negative groups, and paired samples t-tests were used to compare between leg device scores (dominant vs non-dominant leg). Stepwise regression was performed for dominant and non-dominant legs separately to determine the level of variance in device scores associated with infection status (univariate), age, gender, hydration and BMI-for age (multivariate). Statistical analysis was conducted in SPSS version 23 (IBM Corp). Results of the cross-sectional analysis are presented in Chapter 5.

3.6.4 Follow-up analysis of physical measures after preventive chemotherapy

At baseline, only the Indurometer and BIS devices detected any between-group differences. Therefore follow-up measures using the Tonometer and SFM were considered redundant and were not included in further analysis. At each time point, Chi-squared tests, Fisher's

exact tests and independent samples t-tests were used to compare antigen positive and negative group characteristics. Paired samples t-tests were used to compare dominant and non-dominant legs. One-way ANOVA was used to compare within-group characteristics over the data collection points. Stepwise regression was performed for dominant and non-dominant legs separately to determine the level of variance in device scores associated with infection status (univariate), age, gender, hydration, and BMI-for age (multivariate). All analyses were performed using SPSS version 23 (IBM Corp).

Chapter 4: Device reliability and moderating factors

The research questions answered in this Chapter are: *'Can devices used to quantify tissue changes occurring in BCRL be used in FRL populations?'*, and *'What moderating factors need to be considered in interpretation of device scores?'* Related research activities and can be viewed in Figure 4.

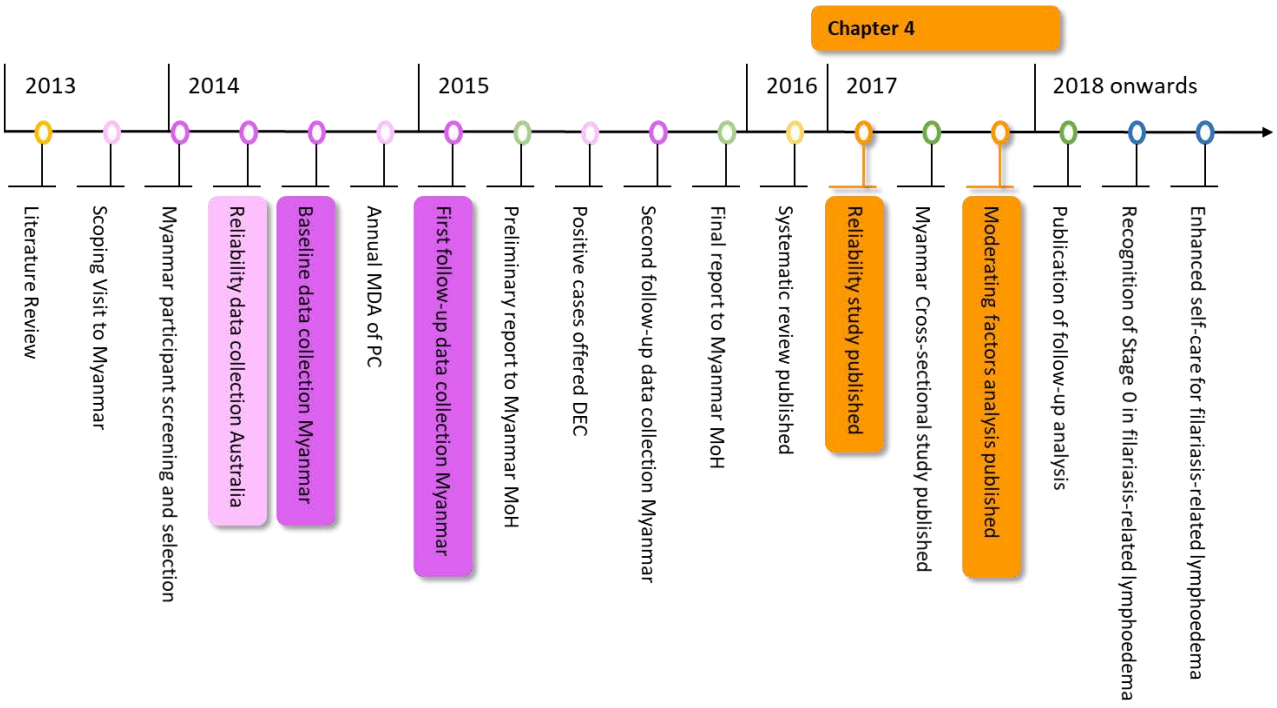


Figure 4: Research activities associated with device reliability and moderating factors in device scores.

4.1 Introduction

Tissue tonometry and BIS have been used extensively to assess unilateral BCRL of the arm in post-menopausal, middle-income women. In this group, few moderating factors other than arm dominance are routinely considered (Dylke et al., 2012). In other limbs or settings additional factors such as age, gender, and systemic hydration may need to be considered when interpreting device scores. Studies on BIS in children and infants report an association between impedance measures, BMI, and age (Avila et al., 2015). No previous investigations of these factors on tissue tonometry have been reported and neither method of assessment had been previously tested in young people in an LF endemic environment. Chapter section 4.2 and 4.3 report the results of reliability and moderating factors analyses on tissue tonometry and BIS respectively. Full text versions of the published paper can be viewed in Appendix F1 and F2.

4.2 Reliability study

This study provided the first peer reviewed report on reliability in tissue tonometry and BIS in the lower limbs of young people in Myanmar and Australia. Result were published in Lymphatic Research and Biology (Douglass, Graves, et al., 2017a). The full text is available in Appendix F1 and Table 4.2 details the contribution of all authors.

Chapter sections 4.2.1, 4.2.2 and 4.2.3 are direct excerpts from the published paper and have been reformatted for the thesis.

Table 4.2: Contribution of authors to ' Intrarater Reliability of Tonometry and Bioimpedance Spectroscopy to Measure Tissue Compressibility and Extracellular Fluid in the Legs of Healthy Young People in Australia and Myanmar'

Author	**Nature and extent of contribution**
Douglass J.	Developed the research question. Recruited participants and collected all data. Performed all data analysis. Wrote the manuscript and developed all figures and tables.
Graves P.	Advised on study design and data analysis strategies. Provided editorial input for the final manuscript.
Gordon S.	Advised on study design and data analysis strategies. Provided editorial input for the final manuscript.

4.2.1 Abstract (abridged)

An intra-rater reliability study was conducted to compare three devices measuring skin and subcutaneous tissue compressibility: a mechanical Tonometer, a digital Indurometer, and a SkinFibroMeter. ECF loads were measured using bio-impedance spectroscopy (BIS). Two populations of tropical-dwelling young people were included: Australian residents in North Queensland aged 8–21 years (n =34) and people aged 10–21 years residing in Central Myanmar (n =38). Neither cohort had any clinical sign of lymphoedema or other leg abnormalities. The mechanical Tonometer and the digital Indurometer had excellent intra-class correlation coefficient (ICC) scores between 0.792 (95% CI 0.055–0.901) and 0.964 (95% CI 0.945–0.984) and the SkinFibroMeter had good to excellent reliability with ICC scores of between 0.565 (95% CI 0.384–0.747) and 0.877 (95% CI 0.815–0.840). BIS exhibited the highest reliability with ICC scores approaching 1.0. These results support the reliable use of tonometry and BIS to assess tissue compressibility and ECF loads in the legs of adolescent populations in developed and developing tropical countries.

4.2.2 Data analysis

The Guidelines for Reporting Reliability and Agreement Studies (Kottner et al., 2011) were used to inform data collection for the reliability analysis. Data collection in Australia was carried out between September 2014 and May 2015. Data collection in Myanmar was during October 2014 and February 2015.

Three device measures were recorded at each measuring point and the average value calculated for data entry. As the data was continuous an intra-class correlation, coefficient (ICC) was used to determine the intra-rater reliability or test-retest reliability. This test indicates the reliability of each device to differentiate participants under repeated, similar

assessment conditions (Kottner et al., 2011). A one-way repeated measure analysis of variance (ANOVA) was used to obtain within subject variance and provide an overall average ICC for each device at each measuring point. Higher ICC scores represent better intra-rater reliability, hence an average score of 0 represents no correlation and 1 equals absolute agreement. A score of less than 0.4 indicates poor agreement between measures, between 0.4 – 0.75 is considered fair to good agreement and a score of more than 0.75 is excellent (Fleiss, 1999).

Each device returns a different unit of measure which prevents direct comparison of device values. To determine the agreement between devices at each measuring location a coefficient of variation (COV) was calculated using the formula:

$$COV = \frac{standard\ deviation \times 100}{Overall\ mean\ for\ the\ device}$$

The COV is reported as a percentage and a lower score indicates less variation between devices (more agreement) in the degree of tissue compressibility at that point (Kottner et al., 2011).

A sample size estimation of 62 subjects was based on an expected correlation coefficient of 0.4 with a 95% confidence interval using an online calculator (Holleran & Ramakrishnan, 2013).

4.2.3 Results

4.2.3.1 Participants

Thirty-four young Australians and thirty-eight young Myanmar people were included in the study. Despite including younger children in the Australian group, there were no significant differences in the mean age, gender, or leg dominance between the two groups. The majority of participants were female (60.4%), the mean age (range) was 15 years (8 – 21) and 95.6% were right leg dominant. The Australian group was significantly taller and heavier than the Myanmar group, but there was no statistically significant difference in BMI (Table 4.2.3.1).

Table 4.2.3.1: Age, height, weight and BMI of participants in Myanmar and Australia.

	Myanmar n = 38			***Australia n= 34***			***t-test***
	Mean ± SD	*Range*	*95% CI*	*Mean*	*Range*	*95% CI*	*p*
Age in years	15.8 ± 3.1	10 - 21	14.8, 16.8	14.7 ± 3.9	8 - 21	13.4, 16.0	0.172
Height in cm*	154.2 ± 9.7	121 - 168	151.1, 157.3	160.2 ± 14.3	130 - 187.5	155.3, 165.1	0.039
Weight in kg*	45.2 ± 9.4	21.3 - 71.2	42.1, 48.2	52.8 ± 16.1	23.2 - 86.1	47.3, 58.3	0.016
BMI	18.8 ± 2.7	13.7 - 27.5	17.9, 19.7	20.0 ± 3.3	12.4 - 28.1	18.9, 21.2	0.088

SD = standard deviation
** significant between group differences*

Indurometry of the anterior thigh and BIS measurements were not recorded for one Australian participant. Poor electrode contact affected a small number of BIS measures and if any single measure was affected, the remaining two measures in that measurement set were also discarded. This resulted in bilateral BIS measures available for 32 Australian and 36 Myanmar participants, with dominant leg values available for all Myanmar people and non-dominant leg values available for 33 Australians.

The midpoint of the anterior thigh recorded the softest measures by all three devices (highest mean values by mechanical Tonometer and Indurometer, lowest mean values by SkinFibroMeter). The hardest tissue was consistently recorded at the midpoint of the calf, and the mean values for the posterior thigh fell between these two ranges for all devices. The BIS ratio of resistance ICF:ECF (Ri:Re) was slightly higher in the non-dominant leg in both populations indicating that this leg had slightly more extracellular fluid than the dominant leg but this was not significant. Mean values and standard deviation for each device at each measuring point are provided as supplementary material (Appendix F1).

4.2.3.2 Intra-class correlation coefficient and coefficient of variation scores

Of the tissue tonometers, the mechanical version (used only in the Myanmar group) had the best ICC (0.893 – 0.964) and COV (10.8% – 20.4%) scores. Excellent ICC scores were also recorded for the Indurometer (0.792 - 0.956) with low (good) COV of 14.8% – 32.2%. The SkinFibroMeter had good to excellent ICC scores (0.565 - 0.877), however this device scored the highest (poorest) COV (18.1% - 43.1%). The ICC scores for BIS approached 1.0 for all measures (ICC = 0.912 - > 0.999) and the COV was very low (2.3% - 20.8%). The ICC, estimated reliability and COV scores for each device and group are reported in Table 4.2.3.2.

Table 4.2.3.2: Intra-class Correlation Coefficient (ICC) and Coefficient of Variation (COV) for each device at all measurement points by population group.

	Myanmar n=38			***Australia n=34***		
	ICC (95%CI)	*COV %*	*n*	*ICC (95%CI)*	*COV %*	*n*
SkinFibroMeter						
Anterior thigh dominant leg	0.640 (0.489, 0.792)	24.2	38	0.659 (0.504, 0.814)	18.1	34
Anterior thigh non-dominant leg	0.877 (0.815, 0.940)	26.1	38	0.699 (0.557, 0.840)	23.5	34
Posterior thigh dominant leg	0.791 (0.691, 0.891)	29.1	38	0.725 (0.593, 0.857)	31	34
Posterior thigh non-dominant leg	0.859 (0.788, 0.930)	43.1	38	0.651 (0.494, 0.809)	25.5	34
Posterior calf dominant leg	0.721 (0.595, 0.847)	38.2	38	0.596 (0.422, 0.769)	25.8	34
Calf non-dominant leg	0.743 (0.624, 0.861)	37.5	38	0.565 (0.384, 0.747)	25.8	34
Indurometer						
Anterior thigh dominant leg	0.900 (0.848, 0.952)	14.8	38	0.792 (0.055, 0.901)	17.8	33
Anterior thigh non-dominant leg	0.882 (0.821, 0.942)	16.6	38	0.909 (0.857, 0.961)	18.35	33
Posterior thigh dominant leg	0.925 (0.886, 0.965)	32.3	38	0.869 (0.798, 0.939)	24.6	34
Posterior thigh non-dominant leg	0.956 (0.933, 0.980)	31.3	38	0.942 (0.909, 0.975)	27.2	34
Posterior calf dominant leg	0.942 (0.910, 0.973)	30.1	38	0.937 (0.901, 0.972)	32.2	34
Calf non-dominant leg	0.906 (0.857, 0.955)	28.9	38	0.921 (0.877, 0.965)	30.5	34
Mechanical Tonometer[1]						
Anterior thigh dominant leg	0.893 (0.838, 0.949)	18.8	38			
Anterior thigh non-dominant leg	0.906 (0.857, 0.955)	11.6	38			
Posterior thigh dominant leg	0.952 (0.013, 0.978)	19.7	38			
Posterior thigh non-dominant leg	0.905 (0.856, 0.955)	18.5	38			
Posterior calf dominant leg	0.964 (0.945, 0.984)	20.4	38			
Calf non-dominant leg	0.927 (0.888, 0.965)	18.4	38			
SBF7						
Re dominant leg	>0.999 (>0.999, >0.999)	2.3	38	>0.999 (>0.999, >0.999)	14.3	32
Ri dominant leg	>0.999 (0.999, >0.999)	20.6	38	0.999 (0.999, >0.999)	27.7	32
Re non-dominant leg	0.999 (0.998, 0.999)	10.0	36	0.999 (0.998, 0.999)	13.5	33
Ri non-dominant leg	0.949 (0.920, 0.978)	20.8	36	0.912 (0.863, 0.962)	27.8	33

1=used in the Myanmar group only

Both the mechanical Tonometer and the Indurometer had better ICC scores when used to measure the posterior thigh and calf than when used to measure the anterior thigh in both populations. ICC scores for the SkinFibroMeter were better in the Myanmar group than the Australian group, but in contrast to the other devices the best ICC scores were on the anterior thigh. The relationship between anatomical location of the measuring points and the ICC score is demonstrated in the radar graph in Figure 4.2.3.2. The data closer to the outside border indicates a higher ICC score, (excellent agreement between repeated measures on a single measurement point) on a scale of 0 to 1.

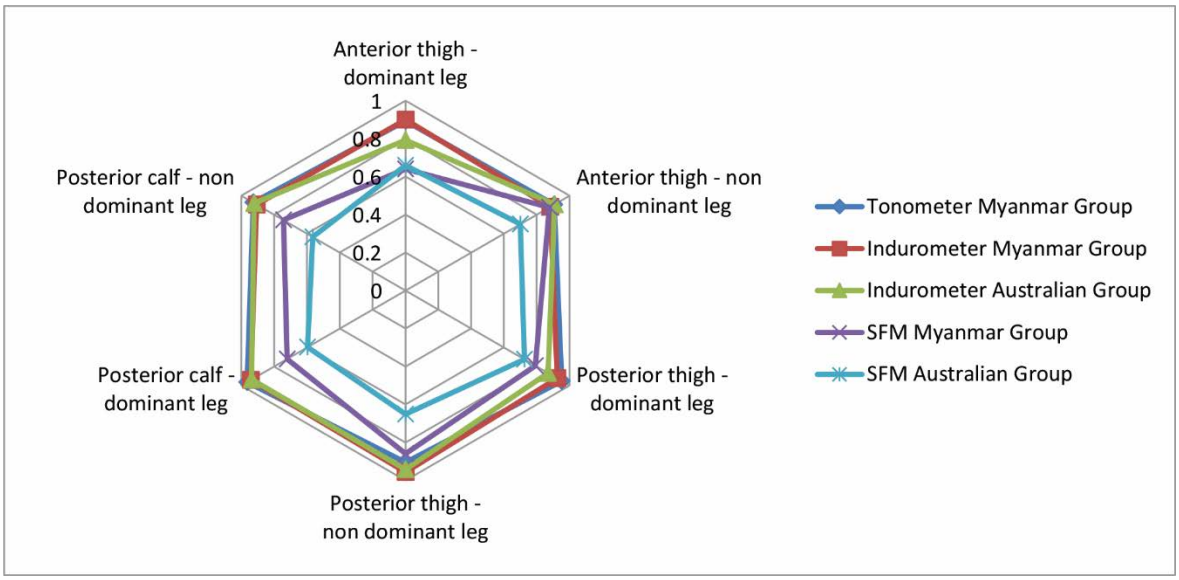


Figure 4.2.3.2: Radar Graph of ICC scores for the three tissue tonometers at six measuring points

The converse was true for the coefficient of variation (COV). All devices recorded the lowest COV (greatest agreement) at the anterior thigh. The relationship between agreement as represented by the COV is demonstrated in Figure 4.2.3.3. Data points closer to the centre represent less difference between the COV (standardized SD as % of mean) of any device's measure at each point, on a scale between 0 and 40%.

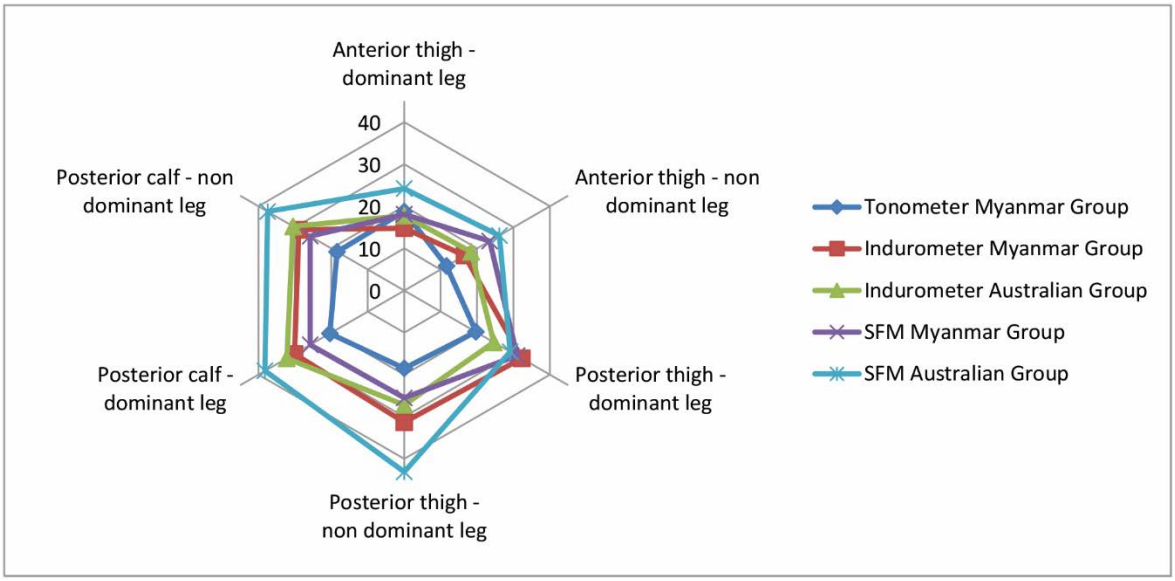


Figure 4.2.3.3: Radar Graph of COV scores for the three tissue tonometers at six measuring points

4.2.4 Summary discussion on device reliability

Reliability analysis on lower limb measures found little difference in ICC scores between devices at any measuring point. The SBF7, Indurometer and Tonometer all demonstrated excellent reliability and the SkinFibroMeter demonstrated good to excellent reliability in the young Australian and Myanmar cohorts.

The first comparison of the mechanical Tonometer and digital Indurometer (Pallotta et al., 2011) reported the coefficient of variation (COV) between three individual operators with less variability when using the Indurometer (range 7% – 16%) than when using the Tonometer (range 7% - 21%). The study population was women with unilateral BCRL and did not report on the affected and unaffected arms separately, but a further study in 2015 on the same devices (Vanderstelt et al., 2015) reported higher COV of between 21.5% (95%CI 19.4–24.1) and 22.8% (95%CI 20.6–25.6) for multiple operators using the Tonometer on the unaffected arm. The lower COV scores (better agreement) for Tonometer measures in the young Myanmar cohort (11.6% - 20.4%) may be due to the use of a single operator who was experienced in device application. There was less variation between operators when using the Indurometer, and unaffected arms (Vanderstelt et al., 2015) returned a COV of between 22.4% and 28.9% which is within the range of scores in the lower limbs of the Myanmar (14.8% – 32.3%) and Australian (17.8% - 32.2%) groups.

As the reliability analysis only compared variance within individual device scores, moderating factors such as gender need not be considered. When using the devices to compare tissue composition between different groups, variables such as age, BMI, and systemic hydration will need to be considered. Therefore, an analysis of device measures to determine which factors should be considered in the cross-sectional and longitudinal analyses was conducted.

4.3 Moderating factors in device measures

This study provided the first peer reviewed report on moderating factors in tissue tonometry and BIS in the lower limbs of young people. A manuscript on the results of the regression analysis on moderating factors associated with variance in device measures in Myanmar and Australia has been accepted for publication in Lymphatic Research and Biology (Douglass, Graves, et al., 2017b). The full text is available in Appendix F2 and Table 4.3 lists the contribution of all authors.

Chapter sections 4.3.1, 4.3.2 and 4.3.3 are direct excerpts from the published paper and have been reformatted for the thesis.

Table 4.3: Contribution of authors to 'Moderating factors in tissue tonometry and bio-impedance spectroscopy measures in the lower extremity of healthy young people in Australia and Myanmar'

Author	**Nature and extent of contribution**
Douglass J.	Developed the research question. Recruited participants and collected all data. Performed all data analysis. Wrote the manuscript and developed all figures and tables.
Graves P.	Advised on study design and data analysis strategies. Provided editorial input for the final manuscript.
Gordon S.	Advised on study design and data analysis strategies. Provided editorial input for the final manuscript.

4.3.1 Abstract (abridged)

A convenience sample of healthy volunteers aged 10 – 21 in Myanmar and 8 – 21 in Australia was recruited. Tissue compressibility at the calf, anterior and posterior thigh was measured using three tonometry devices and free fluid in each leg was assessed using bio-impedance spectroscopy. Data were collected about possible modifiers: leg dominance, age, gender, body mass index (BMI), hydration, and menstrual cycle. Paired t-test and linear regression compared the objective measures with possible modifiers within each population. Statistical significance was set at $p<0.05$ with a 95% confidence interval. In Myanmar, increases in free fluid, tissue compressibility, and limb circumference were associated with being older, female, underweight, or in the second half of the menstrual cycle. In young Australians, increases in tissue compressibility and limb circumference were associated with being older or in the second half of the menstrual cycle.

When assessing tissue compressibility and free fluid in young people using tonometry and BIS, limb dominance and BMI should be considered in a local context and attempts should be made to minimize the potential influences of hydration and the female menstrual cycle.

4.3.2 Analysis of moderating factors associated with variance in measures

Data from all the Australian participants and from the Myanmar participants who tested negative by ICT at baseline were included in this analysis. Growth charts for adolescents in the USA (CDC, 2015) were used to identify underweight (in or below the 5th percentile) or overweight (in or above 85th percentile) participants. Charts were accessed online during June 2016 (CDC, 2015). The effect of age was evaluated by dividing participants into younger (8 – 16 years) or older (17 – 21 years) age groups. In Australia, the average of three measures was recorded for all devices. In the Myanmar cohort, an average of either three or two measures was used dependent on whether the participant had been included in a previous reliability study of the devices (3 measures) or not (2 measures). Between-country comparisons were made using independent samples T-tests for continuous variables and Chi-Square or Fisher's exact tests for categorical variables. Paired samples T-tests were used for between-leg comparisons (dominant vs non-dominant).

Linear regression was used to determine how moderating factors were associated with variance in scores. Due to the number of factors under investigation, the regression was repeated to produce a model with as few variables as possible by removing one insignificant factor at a time. Only factors which significantly predicted variance in scores have been reported. All analysis was completed using SPSS Version 23 (IBM Corp, USA).

4.3.3 Results

4.3.3.1 Participants

All the Australian volunteers and the uninfected participants in Myanmar at baseline were included in this analysis (n=85). Table 4.3.3 shows the characteristics of the Myanmar and Australian cohorts. The mean age was 15.18 years (SD 3.66, range 8 - 21) and there were more females in each cohort: 57% in Myanmar and 60% in Australia. Mean height was 155.43 cm (SD 13.15, range 118.80 - 187.50 cm) and mean weight was 46.5 kg (SD 13.7, range 17.5 - 86.1 kg). Australian participants were significantly taller and heavier than the Myanmar cohort and did not differ markedly from US anthropometric data. A greater portion of Myanmar participants were in or below the 5th percentile for BMI, n=16 (31.4%), compared to Australians, n= 3 (8.8%) but this was only statistically significant between the male cohorts. There were no overweight cases in either group and both cohorts were right leg dominant, Myanmar 98% and Australia 94%. Fewer people in the Myanmar cohort had consumed a drink within the previous hour than their Australian counterparts (26.5% vs 60.6%). There were no significant between-country differences in age groups or time since the last menstrual period (females).

Table 4.3.3.1: Comparison of participant characteristics in Myanmar and Australian cohorts.

Characteristic	Gender	Myanmar n=51	Australia n=34	p=
Older aged group (17 – 21 years) n= (%)	Male	6 (27.3%)	5 (35.7%)	0.604
	Female	13 (44.8%)	9 (45.0%)	0.991
Height Mean cm (SD)	Male	151.40 (13.97)	158.96 (20.97)	0.189
	Female*	152.88 (9.13)	161.08 (9.03)	0.003
Weight in kg Mean kg (SD)	Male*	40.77 (10.39)	51.92 (20.27)	0.037
	Female*	43.40 (9.67)	53.38 (12.94)	0.003
BMI kg/m^2 Mean (SD)	Male*	17.46 (2.41)	19.62 (3.42)	0.033
	Female*	18.43 (2.83)	20.29 (3.31)	0.041
BMI ≤ 5^{th} percentile^ n= (%)	Male*	12 (54.5%)	1 (7.1%)	0.004
	Female	4 (13.8%)	2 (10.0%)	0.527
Last drink > 1 hour before n= (%)	Male	13 (59.1%)	5 (35.7%)	0.194
	Female*	23 (79.3%)	8 (40.0%)	0.002
Last menses < 14 days prior n= (%)	Female	10 (34.5%)	8 (40.0%)	0.672

**significant between group differences*
BMI=body mass index
^CDC growth charts for children in USA accessed online (CDC, 2015)

4.3.3.2 Devices, measuring points and leg dominance

There was a consistent pattern of tissue compressibility at the measurement sites that held true for all devices and all sub groups by age, gender, or infection status. The most compressible (softest) tissue was over the anterior thighs and least compressible (stiffest) tissue was at the calves. Values for the posterior thighs fell between the two and this pattern held true for age, gender, and country subgroups. There was significantly more free fluid in the dominant leg (7%, p=0.034), but this pattern did not hold true for subgroups by country and gender. In both cohorts being in the older age group was associated with larger limb circumference and being underweight was associated with smaller limb circumference.

In the Myanmar cohort, the skin was less compressible (stiffer) over the anterior thigh compared to other sites and thigh circumference was larger on the dominant side. On the non-dominant leg, the skin was less compressible over the posterior thigh and calf, calf circumference was larger, and there was slightly more free fluid. In the Australian cohort, the circumference of the thigh and calf were both slightly larger in the dominant leg with significantly more free fluid on that side (20.7%, p <0.01), but no between-limb pattern of tissue compressibility. Mean device values and between-limb differences for Myanmar and Australian cohorts are provided in Appendix F2.

4.3.3.2.1 Tonometer

Used only in the Myanmar group, increased tissue compressibility at the anterior thigh was associated with being in the older age group and at all posterior limb points with being female or in the second half of the menstrual cycle. Increased tissue stiffness at all posterior limb points was associated with less recent hydration. Table 4.3.3.2.1 shows factors significantly associated with Tonometer measures in the Myanmar group.

Table 4.3.3.2.1: Factors associated with variation in measures using the Tonometer in Myanmar

Measure	***Factor***	***Unstandardized Coefficient (95%CI)***	***Direction***	***t=***
Anterior thigh				
Dominant	17 – 21 years	0.921 (0.466, 1.376)	Softer	4.065**
Non-dominant	17 – 21 years	0.504 (0.056, 0.953)	Softer	2.264*
	Female	0.538 (0.101, 0.976)	Softer	2.474*
Posterior thigh				
Dominant	> 1hr since last drink	-0.891 (-1.494, -0.288)	Harder	-2.976**
	>14 days since menses	0.883 (0.541, 1.226)	Softer	5.197**
Non-dominant	Female	0.998 (0.484, 1.511)	Softer	3.911**
	> 1hr since last drink	-0.895 (-1470, 0.319)	Harder	-3.129**
Calf				
Dominant	Female	1.290 (0.844, 1.736)	Softer	5.824**
	> 1hr since last drink	-0.674 (-1.174, -0.174)	Harder	-2.715**
Non-dominant	Female	1.042 (0.629, 1.456)	Softer	5.077**
	> 1hr since last drink	-0.636 (-1.099, -0.172)	Harder	-2.762**

** p < 0.05 ** p < 0.01*

4.3.3.2.2 Indurometer

In the Myanmar cohort, increased compressibility at the dominant anterior thigh was associated with being in the older age group. For all other measures; being female was associated with increased tissue compressibility and less recent hydration was associated with greater tissue stiffness. There was a significant association with being underweight and increased compressibility at the non-dominant calf. In the Australian cohort, being in the older age group was associated with increased compressibility of both the dominant and non-dominant posterior thighs and with being underweight at both posterior thighs and both calves. Significant factors associated with Indurometer measures are given in Table 4.3.3.2.2.

Table 4.3.3.2.2: Factors significantly associated with variation in measures using the Indurometer

Country/ measure	*Factor*	*Unstandardized Coefficient (95%CI)*	*Direction*	*t=*
Myanmar				
Anterior thigh				
Dominant	17 – 21 years	0.636 (0.276, 0.996)	Softer	3.548**
Non-dominant	Female	0.424 (0.039, 0.810)	Softer	2.216*
	> 1hr since last drink	-0.420 (0.852, 0.013)	Harder	-1.954*
Posterior thigh				
Dominant	Female	0.866 (0.419, 1.312)	Softer	3.900**
	> 1hr since last drink	-0.668 (-1.169, -0.167)	Harder	-2.685*
Non-dominant	Female	0.962 (0.483, 1.441)	Softer	4.042**
	> 1hr since last drink	-0.777 (-1.314, -0.240)	Harder	-2.912**
Calf				
Dominant	Female	0.781 (0.448, 1.113)	Softer	4.725**
	> 1hr since last drink	-0.419 (-0.792, -0.046)	Harder	-2.263*
Non-dominant	Female	0.898 (0.559, 1.237)	Softer	5.331**
	> 1hr since last drink	-0.606 (-0.954, -0.257)	Harder	-3.499**
	< 5^{th} percentile for BMI	0.526 (0.169, 0.882)	Softer	2.970**
Australia				
Posterior thigh				
Dominant	17 – 21 years	0.788 (0.215, 1.360)	Softer	2.804**
	< 5th percentile for BMI	1.281 (0.287, 2.274)	Softer	2.628*
Non-dominant	17 – 21 years	1.126 (0.559, 1.692)	Softer	4.054**
	< 5th percentile for BMI	1.220 (0.237, 2.202)	Softer	2.531*
Calf				
Dominant	< 5^{th} percentile for BMI	1.583 (0.635, 2.531)	Softer	3.400**
Non-dominant	< 5^{th} percentile for BMI	1.103 (0.137, 5.068)	Softer	2.326*

** p < 0.05*

*** p < 0.01*

4.3.3.2.3 SkinFibroMeter

In the Myanmar cohort, increased tissue compressibility at all measurement sites was associated with being female or in the second half of the menstrual cycle, and at the dominant anterior thigh with being in the older age group (17 – 21 years). At both calves, increased tissue stiffness was associated with not having a drink in the previous hour. In the Australian cohort, increased compressibility at the non-dominant anterior thigh and both posterior thighs was associated with being in the older age group and being female was associated with more stiffness at the non-dominant anterior thigh site, but the effect size was small (based on mean values found in supplementary table A in Appendix F3). Significant factors associated with SkinFibroMeter measures are given in Table 4.3.3.2.3.

Table 4.3.3.2.3: Factors significantly associated with variation in measures using the SkinFibroMeter.

Country/ measure	**Factor**	**Unstandardized Coefficient (95%CI)**	**Direction**	**t=**
Myanmar				
Anterior thigh				
Dominant	17 – 21 years	-0.009 (-0.016, -0.002)	Softer	-2.607*
	Female	-0.012 (-0.018, -0.005)	Softer	-3.455**
Non-dominant	>14 days since menses	-0.010 (9-0.014, -0.006)	Softer	-4.972**
Posterior thigh				
Dominant	Female	-0.026 (-0.036, -0.016)	Softer	-5.151**
Non-dominant	Female	-0.019 (-0.032, -0.007)	Softer	-3.109**
Calf				
Dominant	Female	-0.024 (-0.041, -0.006)	Softer	-2.746**
	> 1hr since last drink	0.024 (0.004, 0.043)	Harder	2.432*
Non-dominant	Female	-0.024 (-0.041, -0.007)	Softer	-2.817**
	> 1hr since last drink	0.019 (0.000, 0.038)	Harder	1.972*
Australia				
Anterior thigh				
Non-dominant	17 – 21years	-0.011 (-0.020, -0.002)	Softer	-2.557*
	Female	0.012 (0.003, 0.020)	Harder	2.642*
Posterior thigh				
Dominant	17 – 21years	-0.020 (-0.036, -0.004)	Softer	-2.559*
Non-dominant	17 – 21years	-0.021 (-0.036, -0.005)	Softer	-2.720*

** p < 0.05*
*** p < 0.01*

4.3.3.2.4 SBF7

In Myanmar, increased free fluid was associated with being in the older age group (dominant leg), or in the second half of the menstrual cycle (non-dominant leg) and lower free fluid loads were associated with less recent hydration in both legs. There were no significant associations between any of the factors and BIS measures in the Australian group. Factors significantly associated with BIS measures are given in Table 4.3.3.2.4.

Table 4.3.3.2.4: Factors associated with variation in measures using BIS in the Myanmar cohort.

Limb	Factor	Unstandardized Coefficient (95%CI)	Direction	t=
Dominant leg	17 – 21 years	-0.342 (-0.575, -0.108)	More	-2.295**
	> 1hr since last drink	0.415 (0.172, 0.658)	Less	3.445**
Non-dominant leg	> 1hr since last drink	0.329 (0.046, 0.612)	Less	2.347*
	>14 days since menses	-0.253 (-0.420, -0.087)	More	-3.065**

** $p < 0.05$ ** $p < 0.01$*

4.3.3.2.5 Circumference

In addition to expected variances in limb circumference associated with age or being underweight, in the Myanmar group being in the second half of the menstrual cycle was associated with a larger circumference at the midpoint of the dominant thigh while a smaller circumference at the same point was associated with less recent hydration. In the Australian group, as well as the expected age and underweight related variances, being in the second half of the menstrual cycles was related to a larger circumference at both thigh and calf. Significant factors associated with limb circumference are given in Table 4.3.3.2.5.

Table 4.3.3.2.5: Factors significantly associated with variation in measures of limb circumference.

Country/ measure	Factor	Unstandardised Coefficient (95%CI)	Direction	t=
Myanmar				
Thigh				
Dominant	17 – 21 years	5.199 (2.625, 7.773)	Larger	4.071**
	< 5th percentile for BMI	-5.244 (-7.869, -2.619)	Smaller	-4.026**
	> 1hr since last drink	-2.779 (-5.318, -0.239)	Smaller	-2.205*
	>14 days since menses	1.770 (0.827, 0.238)	Larger	-2.140*
Non-dominant	17 – 21 years	6.444 (4.190, 8.698)	Larger	5.749**
	< 5th percentile for BMI	-6.470 (-8.819, -4.122)	Smaller	-5.540**
Calf				
Dominant	17 – 21 years	2.577 (1.348, 3.806)	Larger	4.217**
	< 5th percentile for BMI	-3.051 (-4.332, -1.771)	Smaller	-4.791**
Non-dominant	17 – 21 years	2.317 (1.113, 3.521)	Larger	3.869**
	< 5th percentile for BMI	-2.936 (-4.191, -1.682)	Smaller	-4.706**
Australia				
Thigh				
Dominant	17 – 21 years	6.746 (2.944, 10.547)	Larger	3.629**
	< 5th percentile for BMI	-11.562 (-17.374, -5.750)	Smaller	-4.069**
	>14 days since menses	2.917 (0.723, 5.110)	Larger	2.720*
Non-dominant	17 – 21 years	6.800 (3.169, 10.430)	Larger	3.830**
	< 5th percentile for BMI	-11.689 (-17.240, -6.137)	Smaller	-4.306**
	>14 days since menses	2.794 (0.699, 4.889)	Larger	2.728*
Calf				
Dominant	17 – 21 years	3.662 (1.458, 5.866)	Larger	3.398**
	< 5th percentile for BMI	-4.973 (-8.343, -1.604)	Smaller	-3.018**
	>14 days since menses	1.509 (0.237, 2.780)	Larger	2.426*
Non-dominant	17 – 21 years	4.033 (1.819, 6.248)	Larger	3.726**
	< 5th percentile for BMI	-5.270 (-8.656, -1.885)	Smaller	-3.184**
	>14 days since menses	1.369 (0.091, 2.646)	Larger	2.191*

** $p < 0.05$ ** $p < 0.01$*

4.4 Summary discussion of moderating factors in device scores

Reliability analysis on the devices found good to excellent intra-rater reliability in measures of healthy young people in Australia and Myanmar, and although each device reports a different unit of measure, the coefficient of variation was low, indicating a good level of agreement at most measurement points.

4.4.1 Patterns of tissue compressibility

Although the units of measure from devices were not directly equivalent, when viewed together they appear as expected for normal tissue composition at each measurement. The fatty layers over the anterior thighs were the most compressible (softest) while the mid-calf point, located over a dense tendo-muscular junction with little overlying fat, was always the least compressible (stiffest), posterior thigh values fell midway between these. This universal pattern of tissue compressibility was maintained regardless of age, gender, or any other sub-grouping.

4.4.2. Variation in device measures

Linear regression for moderating factors demonstrated country specific effects of age, gender, BMI, hydration, and menstrual cycle on tissue compressibility and free fluid loads in the lower limbs of young people. Some factors such as variation in participant hydration may be minimised by administering a drink during the hour prior to measurement and others like the menstrual cycle of young women may depend on composition of the study population. Definitions of leg dominance and parameters for normal BMI should be determined in the context of local life whenever possible.

4.4.2.1. Variation in device measures associated with growth

Healthy, prepubescent children in all countries will undergo noticeable changes in body size and composition as they transition through adolescence and become young adults. In keeping with this expectation, limb circumference in both cohorts increased in an unremarkable association with age and decreased in those who were underweight.

Mechanical Tonometry, which was used in both the Myanmar group and the PNG study, found softer tissues in the Myanmar group, while the Indurometer, which was used in the Myanmar and Australian groups, found softer tissues in the Australian group.

4.4.2.2. Variation in device measures associated leg dominance

There were significant directional changes associated with limb dominance in most measures and in the Myanmar cohort, this was consistent with expected muscle use during a kick. The kicking leg, labelled as dominant, had a slightly larger thigh circumference while the leg supporting the weight of the body to propel it forward during the kick (non-dominant leg), had a slightly larger calf circumference. Between-limb differences in tissue compressibility among Myanmar participants supported this pattern of muscle development (stiffer tissue over the front of the kicking leg and over the back of the supporting leg), however there was no between-leg difference in free fluid loads. Taken together, between-leg measures suggest that neither lower limb is substantially more developed than the other in this cohort.

In contrast, young Australians appeared to be more homolateral with slightly larger thigh and calf circumferences and significantly more free fluid - all on the dominant side, but with no clear pattern of tissue compressibility. The participants in the PNG study also demonstrated the same between-leg, “kicking” pattern, of tissue compressibility and

circumference measures as found in the Myanmar cohort (larger/stiffer dominant thigh, larger/stiffer non-dominant calf). The small difference in between-leg free fluid ratios were similar, but a different variable (ECF only) was used in the PNG cohort so the results are not directly comparable (Gordon et al., 2011). In the PNG group using BIS, between-leg differences in ECF values in the LF negative cases were consistent with the increased free fluid (lower Ri:Re values) found in the dominant leg in the Australian group, but not with the very small shift in the opposite direction among the young Myanmar participants.

It is not possible from the data available to determine exactly why the between-leg patterns are not consistent across all cohorts, perhaps the young Myanmar and PNG people had less access to indoor screen time and had spent more time outside kicking a ball than their Australian counterparts.

4.4.2.3 Variation in device measures associated with being female

There were few universal patterns in the regression models, but having higher tissue compressibility and lower free fluid was associated with being female for all devices at all measuring points. There were also distinct trends toward an association between a larger, softer limb and more free fluid during the second half of the menstrual cycle.

4.4.2.4 Variation in device measures associated with being underweight

Significant associations with being underweight and increased tissue compressibility were found when using the Indurometer, but this was at all posterior points in the Australian cohort and only at the non-dominant calf in the Myanmar cohort. In the absence of standardised growth data for young Myanmar people, using CDC growth charts may have

misclassified some young Myanmar people of normal BMI (for Myanmar) to the underweight category (n = 16); and given the small number of Australians who were classified as underweight (n = 3), reported variances associated with being underweight in either cohort should be viewed cautiously.

4.4.2.5 Variation in device measures associated with hydration and environmental factors

Moderating factors were more frequently associated with variation in device measures in the Myanmar group than among the young Australians. In particular, there were multiple associations with hydration in Myanmar but none in the Australian cohort who were more recently hydrated. Wherever correlations with hydration were found, not having a drink within the previous hour was associated with increased tissue stiffness.

4.4.2.6 Study limitations

In the present study, it was not possible to control for ambient temperatures, but the study sites were roughly equidistant from the equator in dry tropical zones with similar climates. Data was collected in a village administration centre in Myanmar without air-conditioning and in an air-conditioned office in Australia and although daily temperatures or relative humidity were not factored into the analysis, conditions were representative of usual health care environments in each location. Further attention to ambient temperatures are addressed in the follow-up analysis on the Myanmar cohort (Chapter 6) and the discussion (Chapter 7).

4.5 Key points

- Devices used to assess arm lymphoedema were used to assess tissue compressibility and free fluid measures in the legs of health young people living in tropical settings in Myanmar and Australia.
- All devices demonstrated good to excellent reliability in the populations of interest.
- Between-leg patterns of tissue compressibility can be expected to reflect habitual muscle use and leg dominance should be defined in the context of local lifestyles.
- Body composition, age, gender, hydration, and the menstrual cycle were all associated with variation in measures.
- These moderating factors should be considered and minimised when using these devices.
- Reported mean values and coefficients may be useful in clinical management and research on young populations at risk of lower limb lymphoedema.
- Largest variation in compressibility was at the calf which may imply that fluctuations in tissue composition at this site are more easily detected than at fattier measurement sites (such as the anterior thigh).
- Based on the outcome of the reliability study, the devices are suitable for use in young Myanmar people living in an LF endemic area.
- Age, gender, body composition and systemic hydration should be considered in the analysis on device measures in Myanmar.

Chapter 5: Myanmar cross-sectional study

The research question answered in this Chapter is '*Can the devices of interest detect covert connective tissue changes in asymptomatic, LF positive cases?'* Related research activities can be viewed in Figure 5.

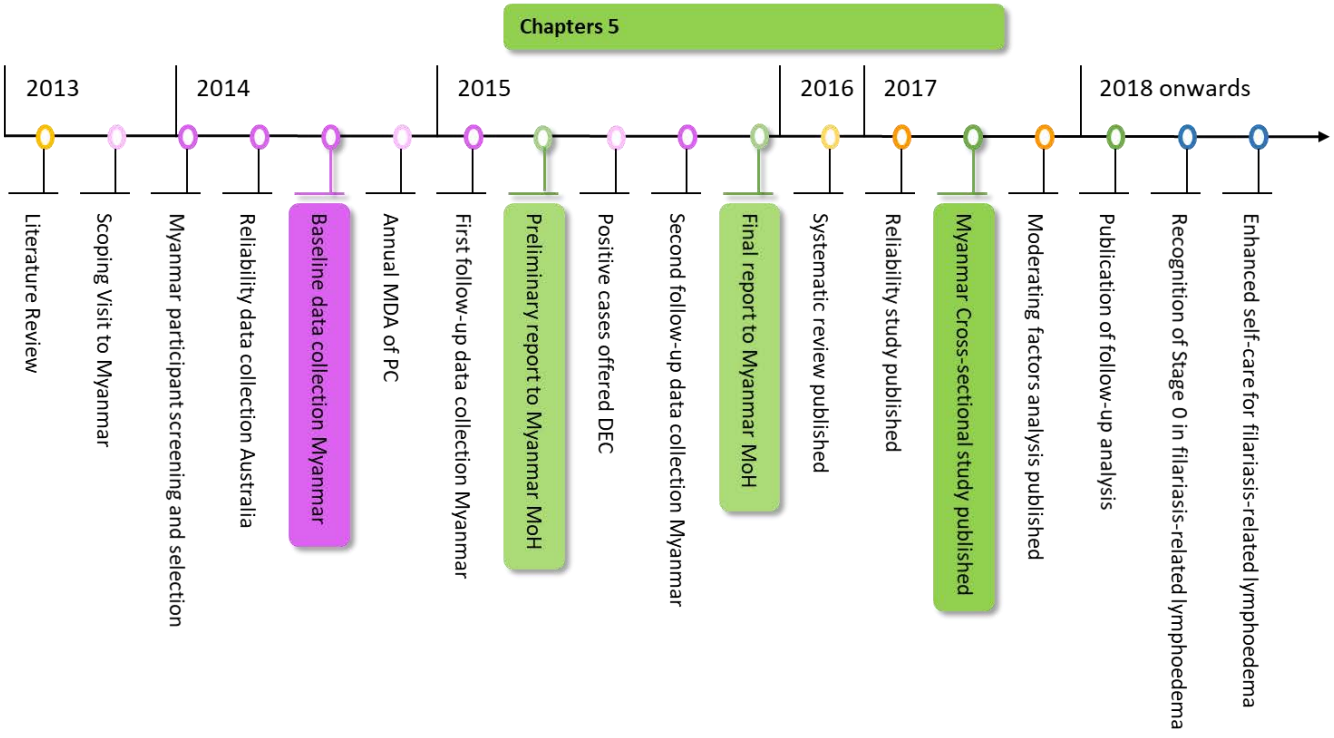


Figure 5: Research activities related to the cross-sectional analysis of baseline data in Myanmar

5.1 Introduction

The preceding chapters have reviewed the advances made in assessment and treatment of lymphoedema after breast-cancer and investigated three tissue tonometers and one BIS device and found them to be reliable the Myanmar cohort. Chapter 5 now explores covert changes in tissue compressibility and free fluid in LF-positive vs LF-negative participants.

This was the first peer reviewed report on covert tissue changes associated with LF-antigenemia in the lower limbs of young people in Myanmar and builds on similar results found in the PNG study. Results of the cross-sectional analysis were published in Tropical Medicine and Infectious Disease in the Special issue "Neglected and Emerging Tropical Diseases in South and Southeast Asia and Northern Australia" (Douglass, Graves, Lindsay, et al., 2017). The full text is available in Appendix CS and Table 5.1 details the contribution of all authors.

Chapter sections 5.2.1 through 5.2.4 are direct excerpts from the published paper and have been reformatted for the thesis.

Table 5.1: Contribution of authors to 'Lymphatic Filariasis Increases Tissue Compressibility and Extracellular Fluid in Lower Limbs of Asymptomatic Young People in Central Myanmar'

Author	**Nature and extent of contribution**
Douglass J.	Developed the study design. Oversaw all participant recruitment and informed consent. Collected data (assisted by Myanmar research assistants). Determined the analysis hypotheses. Performed descriptive analysis. Prepared the manuscript and all figures and tables.
Lindsay, Daniel	Performed statistical analysis. Provided editorial input into the final manuscript.
Graves, Patricia	Advised on study design and data analysis strategies. Provided editorial input into the final manuscript.
Becker, Luke	Trained research assistant in ICT techniques. Selected longitudinal study participants. Provided editorial input into the final manuscript.
Roineau, Maureen	Trained laboratory technicians in the Og4C3 analysis. Provided editorial input into the final manuscript
Masson, Jesse	Performed lab analyses to classify LF infection. Provided editorial input into the final manuscript
Aye, Ni Ni	Provided editorial input into the final manuscript.
Win, San San	Interviewed study volunteers to determine eligibility. Provided editorial input into the final manuscript.
Wai, Tint	Interviewed study volunteers to determine eligibility. Assisted in ICT screening and blood collection activities. Provided editorial input into the final manuscript.
Win, Yi Yi	Interviewed study volunteers to determine eligibility. Provided editorial input into the final manuscript.
Gordon S.	Advised on study design and data analysis strategies. Provided editorial input into the final manuscript.

5.1.1 Reports to the Myanmar Ministry of Health and Sports

A preliminary summary of ICT results and participant characteristics was provided to the Myanmar Ministry of Health and Sports (MOHS) in December 2014 and can be viewed in Appendix A1. Appendix A2 is the final report provided to Myanmar MOHS in 2015 which also includes a preliminary report on the morbidity survey conducted by Ben Dickson in Mandalay Region during January and February 2015.

5.1.2 Data analysis

LF antigen positive cases were defined as those who were positive by either antigen test (ICT or Og4C3 assay). Using WHO growth charts and definitions, underweight participants were identified as being more than two standard deviations below the median BMI-for-age (WHO, 2009). Systemic hydration was approximated by the length of time since the last drink with less than or more than 60 minutes defining the more- or less- hydrated group respectively. Chi-squared tests, Fisher's exact tests, and independent sample t-tests were used to compare antigen positive and negative groups at baseline. Paired samples t-tests were used to compare device measures between dominant and non-dominant legs.

Clinically relevant difference for tonometry measures was set at >10% and for BIS measures at >3%. Stepwise regression was performed for dominant and non-dominant legs separately to determine the level of variance in device measures associated with infection status (univariate) and other potential moderating factors (multivariate). A sample size of 32 in each group was predicted to detect a 10% difference between groups with 80% power based on a mean midcalf value of 2.5 with SD of 0.7 using the Indurometer. (Douglass, Graves, et al., 2017a). Statistical analysis was conducted in SPSS version 23 (IBM Corp) and significance was set at 0.05 with a 95% confidence interval.

5.2 Results of the cross-sectional analysis

5.2.1 Recruitment and participant screening

Screening for LF in Amarapura Township found 60 antigen positive cases among 316 volunteers. Of these, 114 young people (57 cases and 57 controls of the same age and gender) were invited to continue in the longitudinal study (see Figure 5.2.1). Ten people either could not be found or refused to return, and 104 participants were available for baseline blood draw and physical measures. Data from six participants were excluded from the final analysis: four were found at a later measure to have been outside the target age range at baseline, one had a prosthetic leg, and another had a heart condition, neither of the last two were disclosed at the screening interview. The final study population was comprised of 46 antigen positive cases detected by ICT plus a further four cases identified as antigen positive by Og4C3 ELISA (n = 50). There were 48 antigen-negative (control) cases.

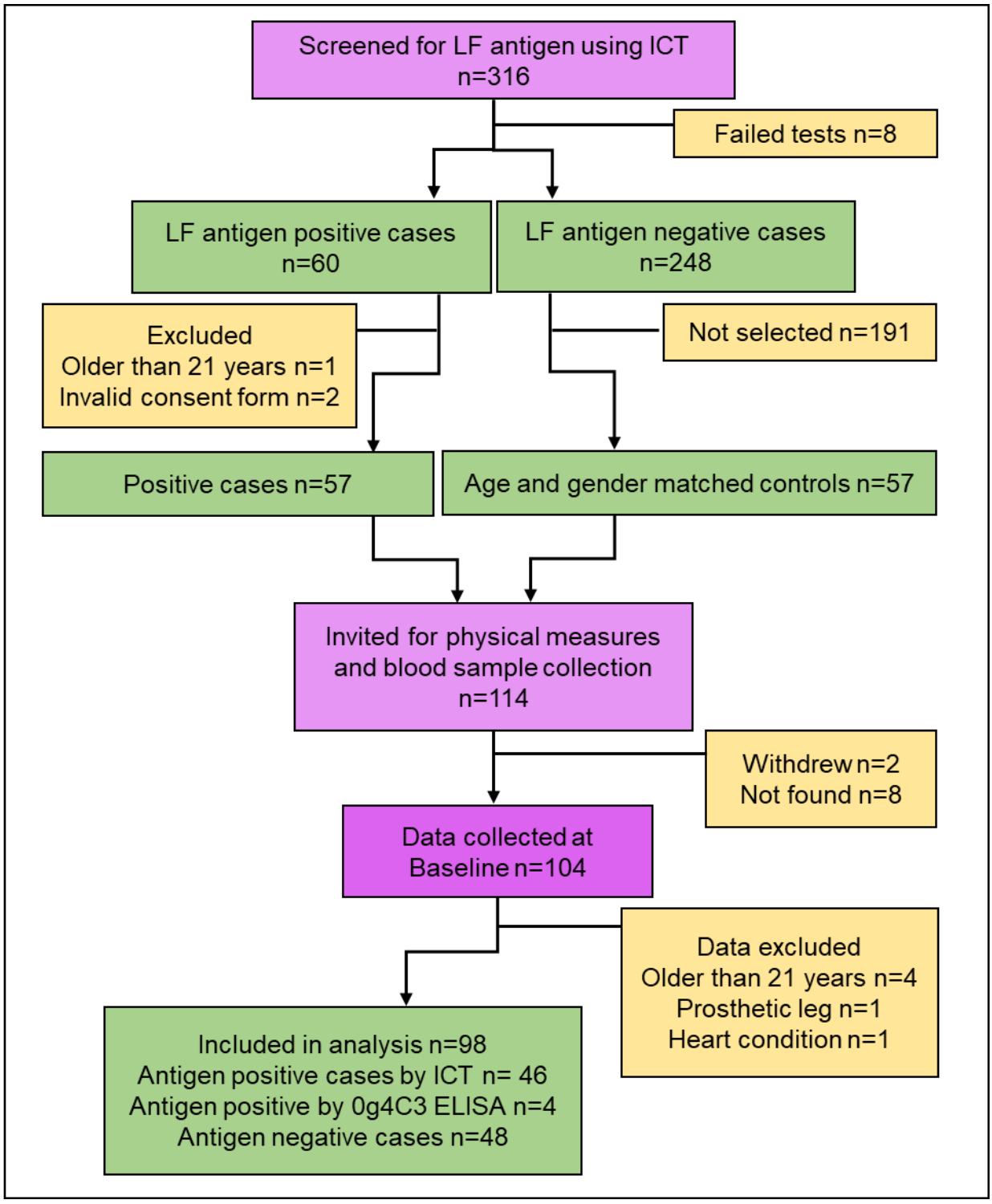


Figure 5.2.1: Flow of participants through recruitment, screening, and baseline data collection.

5.2.2 Participant characteristics

All participants (n = 98) were aged between 10 and 21 years (mean 15.3 SD 3.4) and there were 55 females and 43 males. The mean height, weight, and BMI were 152.0 cm (SD 12.0, range 118.8 – 174.0), 42.3 kg (SD 11.5, range 17.5 – 82.7), and 18.0 kg/m^2 (SD 3.0, range 12.4 – 29.7), respectively. The cohort was 95.9% right leg dominant and 13.3% (n = 13) were considered underweight. Almost half (44.9%) of the participants were working in weaving workshops, 27.6% were students, 8.2% were street vendors, 2.0% were construction workers, and the remaining 17.3% worked in other occupations or did not disclose their occupation. None had a history of lymphoedema in their immediate family, previous surgery, or medical implants, and all were in good health. Two participants were taking prescription medications and one was using traditional medicine. One participant felt unwell on the day scheduled for taking the measures and was asked to return when they felt better. Comparing antigen positive and antigen negative groups there were no significant between-group differences for any physical attribute or moderating factor. Participant characteristics at baseline are shown in Table 5.2.2.1.

Table 5.2.2.1: Group characteristics of antigen positive and antigen negative participants (positive by either immuno-chromatographic test (ICT) or Og4C3) at baseline.

	LF Antigen Positive Cases ***n* = 50**	**Antigen Negative Contr(*n* = 48**	**Mean Diff (95% CI)**	***p* =**
Age in years—mean (SD)	15.20 (3.38)	15.48 (3.46)	0.28 (−1.09, 1.07)	0.691 [a]
Gender				
Female *n* (%)	27 (54%)	28 (58%)		0.410 [b]
Male *n* (%)	23 (46%)	20 (42%)		0.410 [b]
Height in cm—mean (SD)	151.80 (12.56)	152.20 (11.52)	0.399 (−4.44, 5.24)	0.870 [a]
Weight in kg—mean (SD)	42.27 (12.81)	42.30 (10.12)	0.028 (−4.617, 4.670)	0.990 [a]
BMI in kg/m^2—mean (SD)	18.05 (3.46)	18.03 (2.65)	−0.012 (−1.239, 1.216)	0.985 [a]
Body composition *n* = (%)				0.976 [c]
Median weight	41 (82%)	40 (83%)		
Underweight > −2SD	7 (14%)	6 (13%)		
Overweight > +1SD	2 (4%)	2 (4%)		
Dominant leg right/left	47/3	47/1		0.324 [b]
Occupation n = (%)				0.395 [c]
Student	14 (28%)	13 (27%)		
Working/other	32/4 (72%)	34/1 (73%)		
Drank liquid *n* = 97				0.590 [c]
<60 min	13 (26%)	12 (26%)		
>60 min	37 (74%)	35 (74%) (1 NA)		
Consumed 2013 MDA *n* (%)	17 (34%)	22 (46%)		0.383 [c]

LF = lymphatic filariasis; BMI = body mass index; SD = standard deviation; [a] Independent samples T-Test; [b] Fishers exact test; [c] Pearson Chi-Square; NA = participant was not asked.

5.2.3. Moderating factors associated with device measures

5.2.3.1. Effect of Infection on Device Measures

In the antigen positive group, tissue compressibility was higher at all measuring points, and there was more free fluid in both legs compared to that of the antigen negative group. Independent t-tests found that, at mid-calf on the non-dominant side, the increase in Indurometer measures was both clinically relevant (11.1%) and statistically significant (p = 0.021). The mechanical Tonometer also showed the largest between-group difference at the non-dominant calf (antigen-positive group 4.8% softer, p = 0.296) but differences using the SkinFibroMeter were too small to be evident at two decimal places.

Whole leg BIS measures also found clinically relevant (>3%) increases in free fluid in both legs with the largest increase on the non-dominant side (dominant, 4.9%, p = 0.220), (non-dominant, 9.2%, p = 0.053). Mean values and between-group differences for the Indurometer and BIS measures are shown in Table 5.2.3.1 and Figure 5.2.3.1 demonstrates the size of the between-group differences in the non-dominant leg. Mean values and between-group differences for all devices including the Tonometer and SkinFibroMeter are given in supplementary tables in Appendix F4.

Table 5.2.3.1 Between- infection group differences for Indurometer and BIS measures, size and direction of variation.

Measurement Point	**Positive *n* = 50**	**Negative *n* = 48**	**Mean Difference (%)**	**Direction in Positive Cases**	***p* =**
Indurometer	**Mean (SD)**	**Mean (SD)**			
Dominant Anterior Thigh	4.80 (0.76)	4.72 (0.69)	0.05 (1.1%)	Softer	0.731
Non-dominant Anterior Thigh	5.10 (0.88)	5.00 (0.69)	0.10 (1.9%)	Softer	0.546
Dominant Posterior Thigh	4.13 (0.93)	4.06 (0.87)	0.07 (1.7%)	Softer	0.701
Non-dominant Posterior Thigh	3.88 (0.83)	3.86 (0.95)	0.02 (0.4%)	Softer	0.933
Dominant Calf	2.91 (0.57)	2.70 (0.68)	0.21 (7.8%)	Softer	0.096
Non-dominant Calf	2.73 (0.65)	2.46 (0.65)	0.27 (11.1%) *,#	Softer	0.021
BIS					
Dominant Leg *n* = 47/45	2.44 (0.46)	2.56 (0.45)	0.12 (4.9%) #	More fluid	0.220
Non-dominant Leg *n* = 46/44	2.62 (0.56)	2.86 (0.59)	0.24 (9.2%) #	More fluid	0.053

*SD = standard deviation; * Significant between group difference p<0.05; # Clinically relevant between-group difference.*

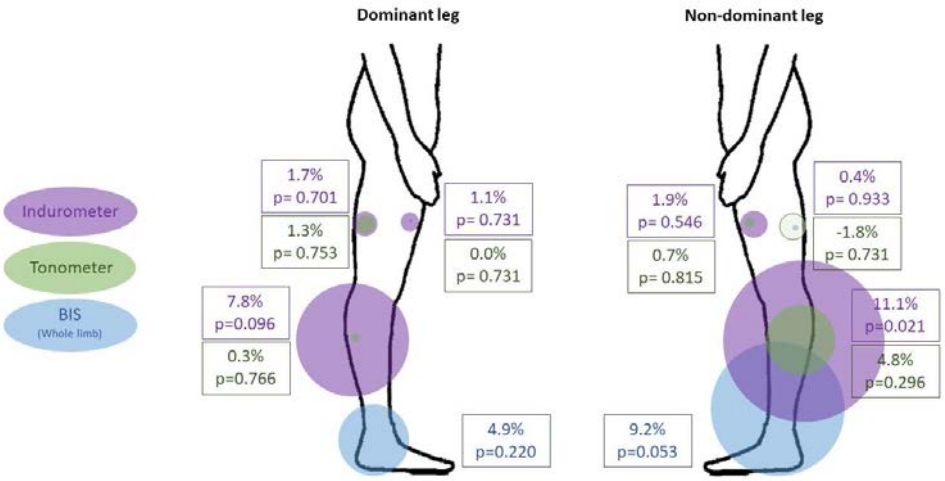


Figure 5.2.3.1 Between infection group differences in tissue compressibility and free-fluid. The size of the circles represents the size of the difference found with each device

5.2.3.2 Effect of all moderating factors on device measures

Regression was first performed with infection status (antigen positivity) alone, and then stepwise regression was used to add moderating factors. Being antigen positive was significantly associated with increased compressibility in the non-dominant calf when using the Indurometer (Table 5.2.3.2, step 1) which is consistent with the t-test results given above in Table 5.2.3.1. Using multivariate regression, after adjustment for other factors (gender, age, underweight, and hydration), increased compressibility remained significantly associated with being antigen positive in the non-dominant calf using the Indurometer, and was also significant in the dominant calf using the same device. When considering all factors, being antigen positive was significantly associated with increased fluid in the non-dominant leg using BIS (Table 5.2.3.2, step 2).

In the stepwise regression, being female was significantly associated with higher tissue compressibility using all three tonometers. The largest gender-related effect using the Indurometer was in calf measures where there is a relatively thin fat layer over the muscles, making small differences in fat and muscle composition more likely to be detected (dominant leg B (SE) = 0.639 (0.117), p <0.000) (see Table 5.2.3.2) The least effect of gender was found over the anterior thighs where the relatively thicker fat layer reduces the influence of the underlying muscle tone and a small difference between the sexes is not likely to register as much change. Using BIS, being female was significantly associated with less free fluid in both legs, and this is consistent with females having relatively smaller muscle/higher fat mass (less fluid) than males of the same age. The largest coefficient was in the non-dominant leg (B (SE) = 0.485 (0.103), p <0.001) (see Table 5.2.3.2).

Table 5.2.3.2: Stepwise regression for moderating factors associated with variation in Indurometer and bio-impedance spectroscopy (BIS) measures.

		Indurometer				**BIS**	
		Higher Values = Increased Tissue Compressibility				**Lower Values = Increased ECF**	
		Posterior Thigh B (SE)		Calf B (SE)		Whole Leg B (SE)	
Factor		Dominant	Non−dominant	Dominant	Non−dominant	Dominant	Non−dominant
Step 1	R^2 =	*0.002*	*0.000*	*0.029*	*0.054*	*0.017*	*0.042*
Antigen Positive		0.070 (0.182)	0.015 (0.180)	0.212 (0.126)	0.272 (0.116) *	−0.117 (0.095)	−0.238 (0.122)
Step 2	R^2 =	*0.189*	*0.187*	*0.283*	*0.269*	*0.283*	*0.398*
Antigen Positive		0.093 (0.168)	0.049 (0.166)	0.234 (0.111) *	0.286 (0.104) **	−0.108 (0.083)	−0.210 (0.099) *
Gender = Female		0.751 (0.178) **	0.679 (0.175) **	0.639 (0.117) **	0.492 (0.110) **	0.230 (0.087) **	0.485 (0.103) **
Older age		0.022 (0.025)	0.041 (0.025)	0.010 (0.017)	0.024 (0.016)	−0.051 (0.012) **	−0.061 (0.015) **
Underweight		0.136 (0.250)	0.277 (0.247)	−0.052 (0.165)	−0.094 (0.155)	−0.237 (0.120)	−0.302 (0.142) *
Less Recent Hydration		−0.338 (0.177)	−0.223 (0.174)	−0.139 (0.117)	−0.239 (0.110) *	0.107 (0.085)	0.124 (0.101)

*ECF = extracellular fluid; SE = standard error; * p <0.05; ** p <0.01.*

Being less well hydrated, defined as not having a drink within one hour of measures, was associated with lower tissue compressibility. This was significant at the non-dominant calf (B (SE) = −0.239 (0.110), p = 0.032). Being older was significantly associated with a small increase in free fluid in both legs, consistent with normal growth increase in muscle mass. Being underweight was significantly associated with a small increase in free fluid in the non-dominant leg (BIS) which may be associated with reduced fat mass or an increased capillary filtrate due to proteinemia.

When accounting for known moderating factors of age, gender, BMI, and hydration, there was a highly significant association between antigen positivity and increased Indurometer measures at the non-dominant calf (p = 0.007). At the dominant calf, the same association was also significant (p = 0.038). When these factors are taken into account for BIS measures, there was a clinically relevant and significant increase in free fluid (Table 5.2.3.2) in the non-dominant leg (p = 0.038).

5.2.4. Patterns of tissue compressibility and free fluid in lower limbs

The overarching pattern of tissue compressibility found in the lower limbs of healthy young people previously reported in the reliability and moderating factors analysis, held true for this cohort which included LF-antigen positive cases. The most compressible tissue was located at the (relatively) fatty anterior thigh, the least compressible tissue was over the dense tendomuscular junction at mid-calf, and values for the posterior thigh fell between the two. When comparing dominant and non-dominant legs, a consistent pattern of between-leg differences was seen and can be attributed to expected muscle activity during a kick. The skin was less compressible (more muscle tone) over the front of the 'dominant' kicking thigh and over the back of the 'non-dominant' thigh and calf muscles which propel the body forward during the kick. Using BIS, there was more free fluid (more muscle mass or less fat) in the dominant leg compared to the non-dominant leg (9.6%); this difference was both clinically relevant (>3%) and statistically significant ($p < 0.01$) using paired samples t-tests. Mean values and between-leg differences for the Indurometer and BIS are given in Table 5.2.4. Mean values and between-leg differences for all devices including the Tonometer and SkinFibroMeter and are given in supplementary tables in Appendix F4.

Table 5.2.4: Mean values and between-leg differences using the Indurometer and BIS.

	Indurometer (*n* = 98)			**BIS (*n* = 90)**
	Anterior Thigh	**Posterior Thigh**	**Calf**	**Whole Leg**
Dominant leg Mean (SD)	4.74 (0.72)	4.10 (0.90)	2.81 (0.63)	2.50 (0.46)
Non–dominant leg Mean (SD)	5.05 (0.79)	3.87 (0.89)	2.60 (0.59)	2.74 (0.59)
Mean difference (SD)	−0.31 (0.31)	0.23 (0.23)	0.21 (0.21)	−0.24 (0.32)
95% CI of the difference	−0.41, −0.21	0.11, 0.35	0.13, 0.28	−0.31, −0.17
% difference	6.5% **	5.6% **	7.5% **	9.6% **,#
Direction (dominant leg)	Harder	Softer	Softer	More fluid

*SD = standard deviation; ** Significant between-leg difference p ≤ 0.01; # Clinically relevant between - leg difference (tonometry >10%, BIS >3%).*

The overall pattern of between-leg differences (dominant vs. non-dominant), as demonstrated by kicking a ball, was maintained in the antigen positive cases, but the degree of difference was altered. Figure 5.2.4 is a radar graph showing the percentage of between-leg differences in Indurometer and BIS values for the whole cohort and by infection group. In the infected group, between-thigh differences in tissue compressibility were exaggerated (closer to the outer ring in the radar chart) but only slightly, with similar percentage differences for positive (7%), negative (6.1%), and whole cohort groups (6.5%). The between-infection group differences were more pronounced at the calf where the mean between-calf difference in the positive cases (6.5%) was much smaller (closer to the middle) than that of the negative cases (9.7%) or whole cohort (7.5%). Similarly, as well as an overall increase in free fluid, BIS results indicated that positive cases had smaller between-leg differences compared to those of their negative counterparts (7.5% vs. 11.7%). Although not statistically significant, these reduced between-leg differences in the distal legs of the antigen positive cases suggest a covert oedema overlying and masking normal between-leg variations in muscle tone and mass.

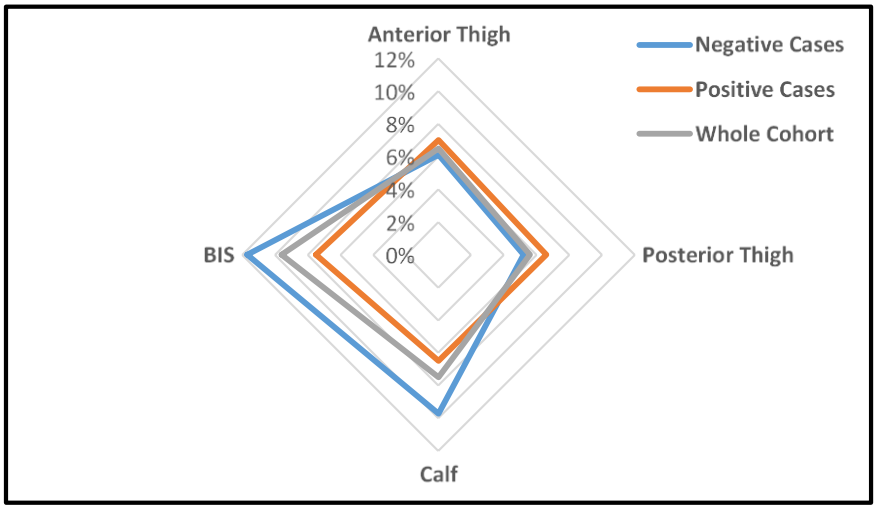


Figure 3: Percentage between-leg differences using the Indurometer and BIS in the LF

Figure 5.2.4: Percentage between-leg differences using the Indurometer and BIS in the LF antigen negative cases, LF antigen positive cases, and whole cohort. Data points which are closer to the outer ring indicate greater between leg-differences.

5.3 Summary discussion on the cross-sectional analysis

Results in the Myanmar study reinforce earlier findings from PNG (Gordon et al., 2011) where clinically significant between-infection group differences were found in physical leg measurements. However, some differences in observations between studies were noted. In particular, among young people in PNG who were LF-positive, increased tissue compressibility was found at the posterior thighs using the mechanical Tonometer. In the Myanmar cohort, the between-infection group differences were found using the digital Indurometer at the calf. There may be several reasons for this discrepancy. The PNG cohort had a higher proportion of females (64% vs. 54%) than the Myanmar cohort and a higher mean BMI (19.7 vs. 18.1). In addition, age, gender, and hydration were not considered in the PNG analysis. In the Myanmar study, the Tonometer returned slightly softer measures in the dominant posterior thigh and non-dominant calf, but in this cohort, the differences were not significant.

Although the only statistically significant between-group difference was by Indurometry, the pattern of Tonometer and BIS scores reinforce a trend towards increased tissue compressibility at the non-dominant calf and increase free fluid on the non=-dominant side.

5.4 Key points

- LF antigenemia is associated with increased tissue compressibility at the calf and with higher free fluid in the non-dominant leg.
- The size and direction of the associations among the antigen-positive cohort are consistent with a covert, high protein, subcutaneous oedema in the distal leg.
- Regression analysis for moderating factors of age, gender, and hydration reinforced the between-infection group differences.
- Further exploration of these low-cost devices in clinical and research settings on FRL is warranted.

Chapter 6: Myanmar longitudinal study

This Chapter provides a descriptive analysis of follow-up data and aims to address the question '*What is the effect of preventive chemotherapy on tissue composition in LF antigen positive cases?*'

Preliminary data analysis revealed that the largest and most significant contribution to variation in device scores was associated with time. Further modelling is required to account for this effect and is being carried out in collaboration with a biostatistician. A manuscript with these results will be submitted to a peer reviewed journal during 2018. Research activities relevant to this chapter can be viewed in Figure 6.

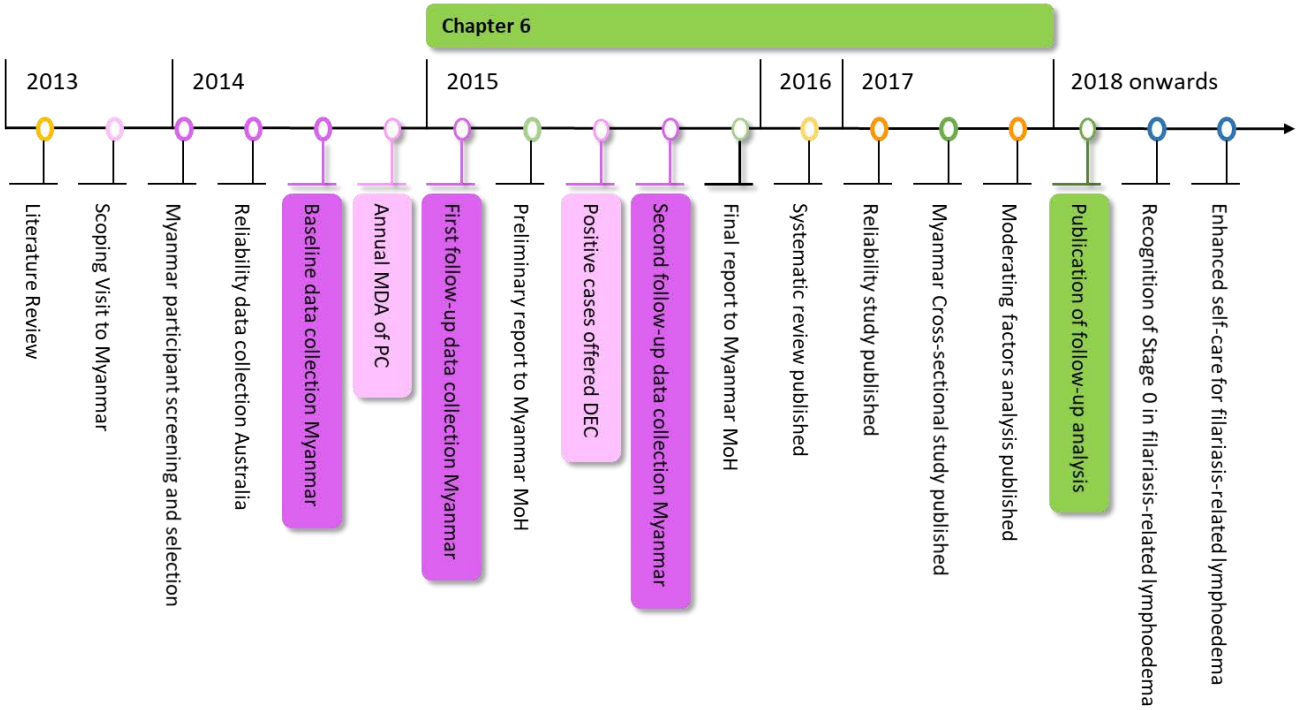


Figure 6: Research activates contributing to a descriptive analysis of the longitudinal data.

6.1 Introduction

This was an investigation on physical measures of covert connective tissue change associated with LF-antigenemia before and after PC. Therefore, only devices which had detected differences between infection groups at baseline (Chapter 5) were included in the longitudinal analysis.

A large variation in the magnitude of scores was of interest even if not statistically significant. Therefore, in addition to reporting statistical significance in absolute measures, clinical importance - defined as the magnitude of change required to be classified as latent pathology- is also reported (Leek et al., 2017). At baseline, a higher clinical percentage was chosen for Indurometer scores (>10%) to minimise overestimating the effect of infection on tissue pathology. At follow up, a lower difference (5%) was set to detect the smallest possible effect of the intervention. Analysis of BIS scores used a >3% criteria for clinical relevance at baseline and follow-up.

6.1.1 Analysis of follow-up data

Variation in participant characteristics between-infection groups at each time point and within-infection groups over the three time-points were compared using t-test and one way ANOVA for continuous variables, and chi-square tests for categorical variables.

6.2 Results

Preliminary analysis of device scores indicated a significant variation across the three measurement times which was not linear (see Figure 6.2.3.1) and therefore could not be explained by natural growth among the participants. As it could not be determined if this was a seasonal effect or any other unmeasured factor, data from Feb-15 were not included in any before-and-after-PC analysis.

6.2.1 Participants

This was a before and after comparative cohort study on participants who were included in the cross-sectional analysis on tissue compressibility and free fluid in the lower limbs reported in Chapter 5 (n = 98) (Douglass, Graves, Lindsay, et al., 2017). Figure 6.2.1. shows the flow of participants through the longitudinal study. Two data sets from participants that tested negative by ICT at baseline were excluded from further analysis when discrepancies in Og4C3 results at follow-up could not be resolved. At the Feb-15 follow-up, eight participants could not be found or declined to return. At the Jun-15 follow-up, four people who had missed the Feb-15 follow-up presented for measures, but 14 others could not be found or did not return for unknown reasons. At Jun-15 follow-up one participant revealed that she was five months pregnant which meant that she had been pregnant during the Feb-15 follow-up measures but not at baseline and her dataset was excluded from the follow-up analysis. This provided 96 participants at baseline, 87 participants at the Feb-15 follow-up and 77 participants at the Jun-15.

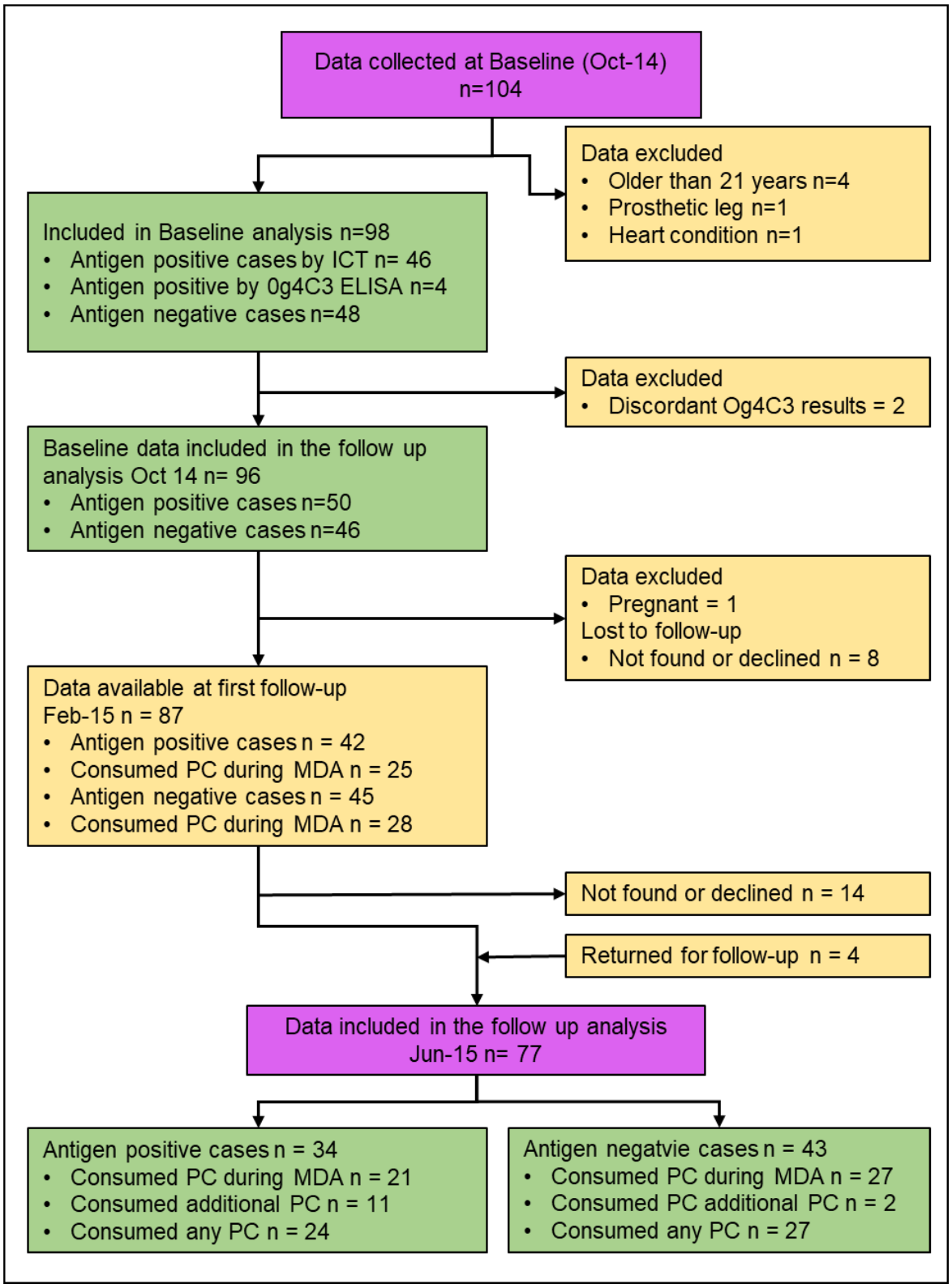


Figure 6.2.1: Flow chart of participants through the longitudinal study

6.2.1.1 Participant characteristics

At baseline (Oct-14), the mean cohort age was 15.3 (SD 3.41) years and 55.2% were female. There were no significant between-infection group differences for age, gender, height, weight, BMI, BMI-for-age, time since the last menstrual period (females), or occupation, and this remained true at both Feb-15 and Jun-15 follow-up. Between-infection group characteristics at each time-point are given in Table 6.2.1.1.

Table 6.2.1.1: Between-infection group characteristics at each time-point

Factor	Time	n =	Ag -	Ag +	P=
Age, mean years (SD)	*Oct-14*	96	15.41 (3.468)	15.20 (3.381)	0.762 a
	Feb-15	87	15.33 (3.30)	14.98 (3.50)	0.625 a
	Jun-15	77	16.28 (3.35)	15.38 (3.68)	0.267 a
Age group, 10-13 years n= (%)	*Oct-14*	34	17 (37.0%)	17 (34.0%)	0.731 b
	Feb-15	30	14 (31.1%)	16 (38.1%)	0.752 b
	Jun-15	23	11 (25.6%)	12 (35.3%)	0.649 b
Age group, 14-17 years n= (%)	*Oct-14*	33	14 (30.4%)	19 (38.0%)	0.731 b
	Feb-15	32	18 (40.0%)	14 (33.3%)	0.752 b
	Jun-15	29	17 (39.5%)	12 (35.3%)	0.649 b
Age group, 18-21 years n= (%)	*Oct-14*	29	15 (32.6%)	14 (28.0%)	0.731 b
	Feb-15	25	13 (28.9%)	12 (28.6%)	0.752 b
	Jun-15	25	15 (34.9%)	10 (29.4%)	0.649 b
Gender, female n= (%)	*Oct-14*	96	26 (56.5%)	27 (50.9%)	0.988 c
	Feb-15	87	27 (64.3%)	23 (54.8%)	0.668 c
	Jun-15	77	28 (65.1%)	22 (64.7%)	1.000 c
Height, mean cm (SD)	*Oct-14*	96	152.55 (11.64)	151.80 (12.56)	0.764 a
	Feb-15	87	153.28 (11.34)	151.56 (12.57)	0.504 a
	Jun-15	77	154.10 (9.58)	149.65 (12.07)	0.075 a
Weight, mean kg (SD)	*Oct-14*	96	42.62 (10.16)	42.27 (12.81)	0.883 a
	Feb-15	87	44.05 (9.81)	43.29 (12.98)	0.758 a
	Jun-15	77	44.61 (9.30)	40.36 (11.83)	0.082 a
BMI, mean kg/m^2 (SD)	*Oct-14*	96	18.04 (2.79)	18.04 (3.33)	0.993 a
	Feb-15	87	18.50 (2.68)	18.40 (3.36)	0.874 a
	Jun-15	77	18.67 (2.78)	17.57 (3.23)	0.113 a
Underweight, n=(%)	*Oct-14*	96	6 (13.0%)	7 (14.0%)	0.988 b
>2SD <median BMI-for-age	*Feb-15*	87	6 (13.3%)	5 (11.9%)	0.979 b
	Jun-15	77	3 (7.0%)	7 (20.6%)	0.204 b
Last menstrual period, n=(%)	*Oct-14*	96	12 (31.6%)	11 (28.9%)	0.932 b
>14 days since last menses	*Feb-15*	87	5 (11.1%)	9 (21.4%)	0.025 b
	Jun-15	77	7 (16.3%)	2 (5.9%)	0.108 b
Last drink	*Oct-14*	96	33 (34.3%)	37 (38.5%)	0.576 c
> 60 minutes since last drink	*Feb-15*	87	26 (57.8%)	27 (64.3%)	0.661 c
	Jun-15	77	17 (39.5%)	14 (41.2%)	1.000 c
Consumed PC, n=(%)	*Feb-15*	85	28 (62.2%)	25 (59.5%)	1.000 c
Either MDA or DEC alone	*Jun-15*	77	27 (52.8%)	24 (70.6%)	0.628 c

a) T-test *b) Pearson Chi-Square* *c) Fishers exact test*

Within-infection group characteristics also did not change significantly over time other than the level of recent hydration. At the Jun-15 follow-up, significantly more people had consumed a drink within the hour prior to measurement in both groups. Within-infection group characteristics at three time-points are given in Table 6.2.1.2.

Table 6.2.1.2: Within-group characteristics at each time-point

	Ag	**Oct-14 n = 96**	**Feb-15 n = 87**	**Jun-15 n = 77**	**P=**
Age, mean years (SD)	*Negative*	15.41 (3.47)	15.33 (3.30)	16.28 (3.35)	0.350 a
	Positive	15.20 (3.38)	14.98 (3.50)	15.38 (3.68)	0.897 a
Age group, 10 - 13 years n = (%)	*Negative*	17 (37.0%)	14 (31.1%)	11 (25.6%)	0.756 b
	Positive	17 (34.0%)	16 (38.1%)	12 (35.3%)	0.992 b
Age group, 14 - 17 years n = (%)	*Negative*	14 (30.4%)	18 (40.0%)	17 (39.5%)	0.756 b
	Positive	19 (38.0%)	14 (33.3%)	12 (35.3%)	0.992 b
Age group, 18 - 21 years n = (%)	*Negative*	15 (32.6%)	13 (28.9%)	15 (34.9%)	0.756 b
	Positive	14 (28%)	12 (28.6%)	10 (29.4%)	0.992 b
Gender, female n= (%)	*Negative*	26 (56.5%)	27 (60.0%)	28 (65.1%)	0.707 b
	Positive	27 (54.0%)	23 (54.8%)	22 (64.7%)	0.579 b
Height, mean cm (SD)	*Negative*	152.55 (11.64)	153.28 (11.34)	154.10 (9.58)	0.798 a
	Positive	151.80 (12.56)	151.56 (12.57)	149.65 (12.07)	0.712 a
Weight, mean kg (SD)	*Negative*	42.62 (10.16)	44.05 (9.81)	44.61 (9.30)	0.612 a
	Positive	42.27 (12.81)	43.29 (12.98)	40.36 (11.83)	0.598 a
BMI, mean kg/m^2 (SD)	*Negative*	18.04 (2.79)	18.50 (2.68)	18.67 (2.78)	0.513 a
	Positive	18.04 (3.33)	18.40 (3.36)	17.57 (3.23)	0.562 a
Underweight, n= (%)	*Negative*	6 (13.0%)	6 (13.3%)	3 (7.0%)	0.889 b
>2SD <median BMI-for-age	*Positive*	7 (14.0%)	5 (11.9%)	7 (20.6%)	0.865 b
Last menstrual period, n= (%)	*Negative*	7 (15.2%)	5 (11.1%)	7 (16.35)	0.597 b
>14 days since last menses	*Positive*	8 (16%)	9 (21.4%)	2 (5.9%)	0.425 b
Last drink	*Negative*	33 (71.7%)	26 (57.8%)	17 (39.5%)	0.016 b
> 60 minutes since last drink	*Positive*	37 (74.0%)	27 (64.3%)	14 (41.2%)*	0.009 b
Consumed PC, n= (%)	*Negative*	n/a	28 (62.2%)	27 (62.8%)	1.000 b
Either MDA or DEC alone	*Positive*	n/a	25 (59.5%)	24 (70.6%)	0.622 b

Ag = antigen status *SD = standard deviation* *BMI = body mass index*
a) Oneway ANOVA *b) Pearson Chi-square* ** p<0.05*

6.2.2 Consumption of preventive chemotherapy

Self-reported PC consumption during the 2013 MDA was 40.1% and in Feb-15 60.9% of returning participants reported that they had taken PC during the 2014 MDA. Between the two MDA events, consumption went up among positive females, but down among positive males. Table 6.2.2 shows the PC consumption for positive and negative groups and by gender. There was no significant between-infection group difference in the proportion of PC consumed during the 2014 MDA (antigen negative 62.2%, antigen positive 59.5%, p=0.80). At the Jun-15 follow-up after the positive cases had been offered further PC, a total of 66.2% of participants had taken PC either during the 2014 MDA or in March 2015.

Table 6.2.2: Self-reported consumption of PC by infection-group and gender.

	2013 MDA	**2014 MDA**	**2014 MDA or DEC**
Positive cases All n = (%)	17 (34.0%)	25 (59.5%)	24 (70.6%)
Males n = (%)	15 (65.2%)	9 (47.4%)	8 (66.7%)
Females n = (%)	2 (7.4%)	16 (69.6%)	16 (72.7%)
Negative cases All n = (%)	22 (47.8%)	28 (62.2%)	27 (62.8%)
Males n = (%)	13 (65.0%)	2 (11.1%)	11 (73.3%)
Females n = (%)	9 (34.6%)	16 (59.3%)	16 (57.1%)

MDA = mass drug administration of Albendazole and DEC *DEC= diethylcarbamazine citrate*

6.2.3 Device measures

6.2.3.1 Tissue compressibility and free fluid in the lower limbs

Figure 6.2.3.1 shows mean cohort values for a) Indurometer and b) BIS scores at each time-point. The consistent, overall pattern of tissue compressibility previously described in Chapters 4 and 5, held true at follow-up. The most compressible tissue (highest Indurometer scores, range 4.31 – 5.16) was found over the anterior thighs while the stiffest tissue (lowest values, range 1.95 – 2.80) was at the mid-calf where there is little underlying fat. Free fluid was higher in the dominant limb (lower BIS scores, range 2.50 – 2.84) at all three time-points.

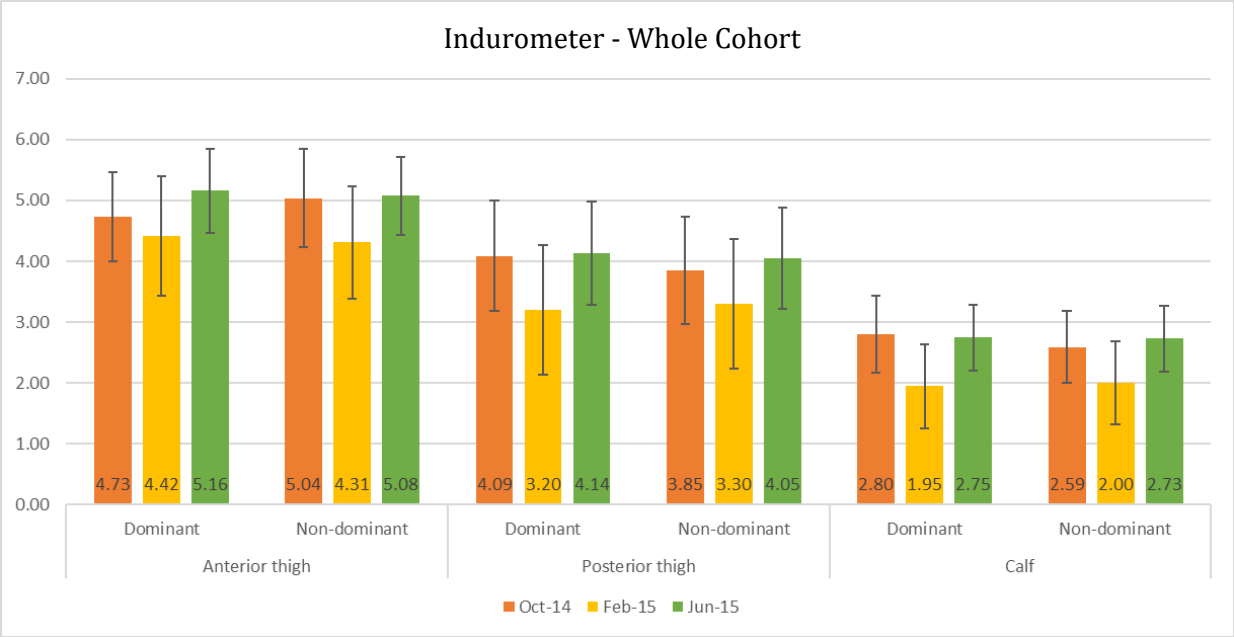


Figure 6.2.3.1: a) Mean Indurometer scores for each measurement site at all three time-points. Higher values = more tissue compressibility.

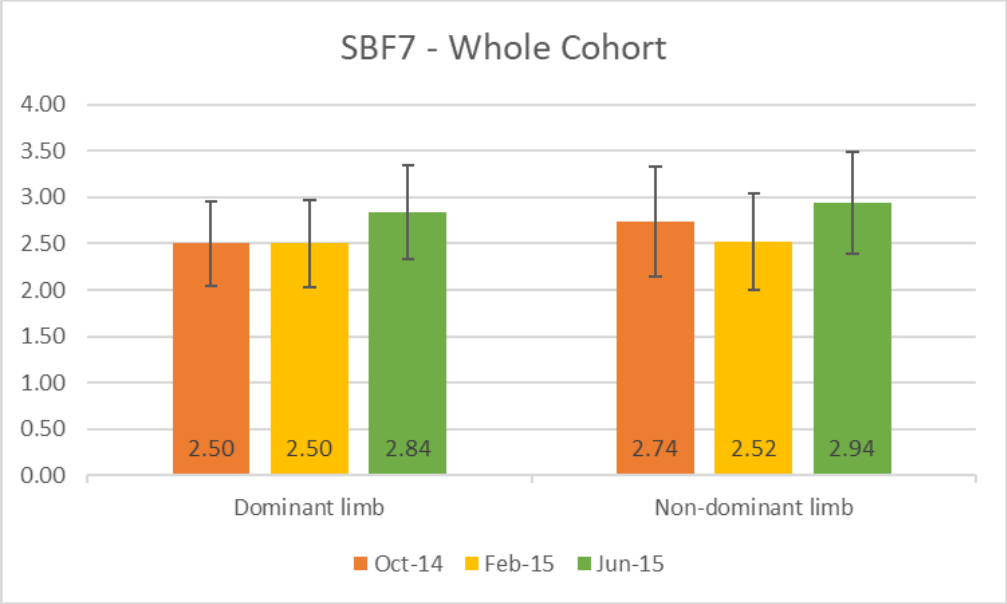


Figure 6.2.3.1: b) Mean BIS scores for each leg at all three time-points. Lower values = more free fluid.

6.2.3.2 Effect of time on device measures

There was a significant non-linear association with time for all measures. At Feb-15, overall tissue compressibility had reduced, but then increased again to higher than baseline at the Jun-15 follow-up ($p<0.000$ for all measures, Fig 6.2.3.1-a). The magnitude of variation was greatest at the dominant calf with 30.4% reduction between baseline and Feb-15 and 41.0% increase from Feb-15 to Jun-15.

Free fluid in the non-dominant limb had increased 8.8% at Feb-15 but by Jun-15 free fluid in both legs had reduced to below baseline measures (dominant leg -13.6%, non-dominant leg -7.3%, $p<0.000$ for all SBF7 measures, Fig 6.2.3.1-b).

6.2.3.3 Effect of LF infection on tissue compressibility

Graphs of unadjusted mean Indurometer scores by infection group at each time point can be seen in Figure 6.2.3.3. At baseline the LF-antigen positive cases had more compressible tissue at all measurement points compared to their antigen negative peers. This appeared to be clinically relevant at both calves (>10%) but was only statistically significant at the non-dominant calf (12.24%, mean difference 0.30 (95%CI 0.531, 0.069), p= 0.011, Fig 6.3.3.3-f).

At Feb-15 the between-infection group differences had reversed. Mean device scores were lower among the LF-antigen positive cases at every measurement point but none of the differences were statistically significant (Figure 6.2.3.3).

At Jun-15 the between-infection group differences were mixed, and none were statistically significant. The largest remaining difference was at the dominant calf (6.7% higher in the antigen positive group p=0.142) but this was not statistically significant. The significant and clinically relevant difference in non-dominant calf scores which had been present at baseline was not present at Jun-15 follow-up (2.6% higher in the antigen positive group, p=0.608) (Figure 6.2.3.3-f).

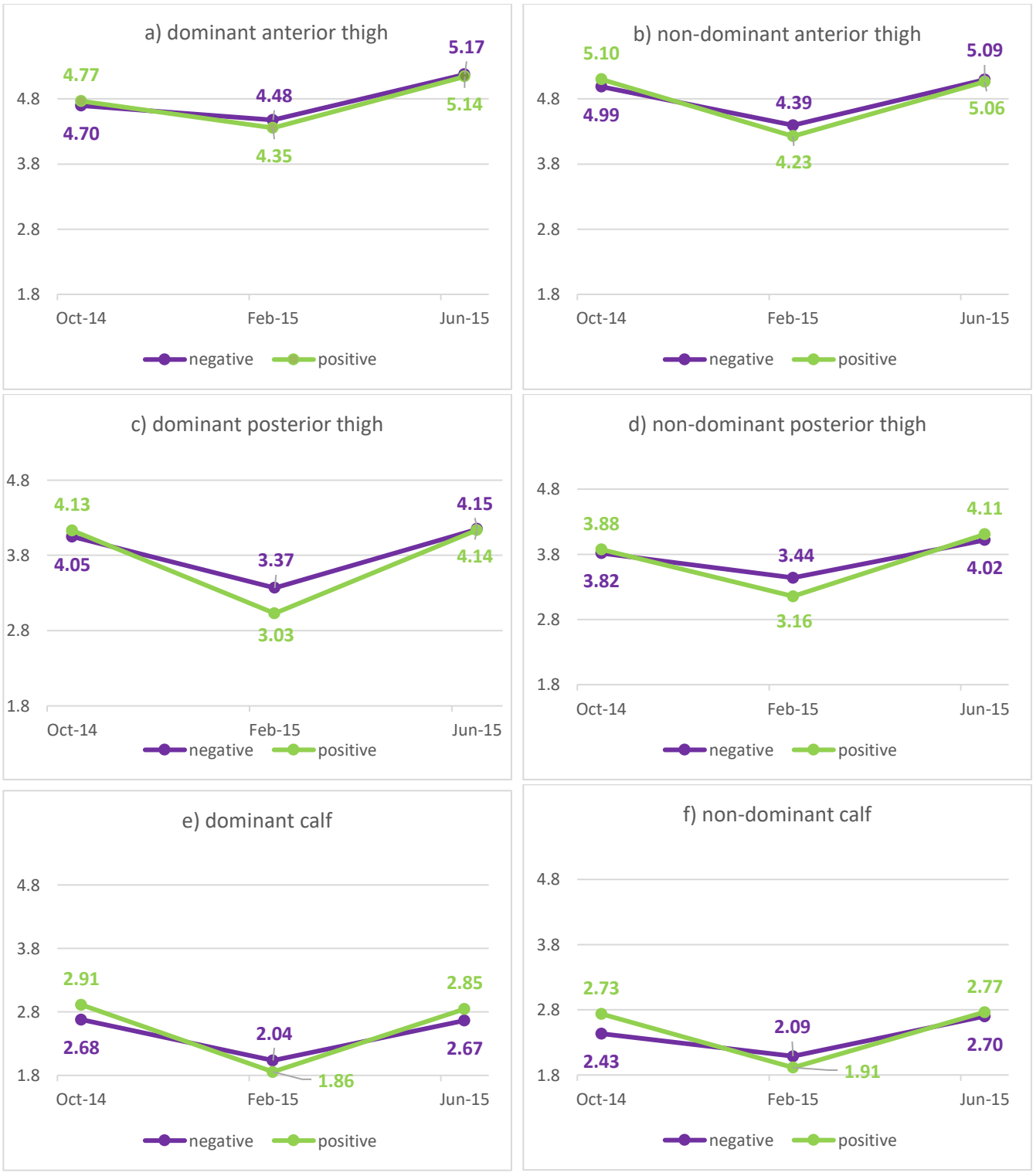


Figure 6.2.3.3: Mean Indurometer scores by infection group over three time-points at a) dominant anterior thigh, b) non-dominant anterior thigh, c) dominant posterior thigh, d) non-dominant posterior thigh, e) dominant calf, f) non-dominant calf.

6.2.3.4 Effect of infection on free fluid

At baseline the LF-antigen positive cases had more free fluid in both lower limbs compared to their antigen negative peers. While this appeared to be a clinically relevant difference (>3%), without adjusting for other moderating factors it was not statistically significant for either limb (non-dominant side 9.1% p= 0.062, dominant side 4.7% p= 0.227). Graphs of unadjusted mean BIS scores by infection group at each time point can be seen in Figure 6.2.3.4.

At Feb-15, the between-infection group differences in free fluid had reduced in the dominant leg below clinical relevance (it disappeared in the non-dominant leg and neither was statistically significant).

At Jun-15 there were only small, non-significant differences in free fluid between the infected and uninfected groups (dominant limb 2.1% more free fluid among negative cases p= 0.644), (non-dominant limb 0.3% more free fluid among positive cases p= 0.928).

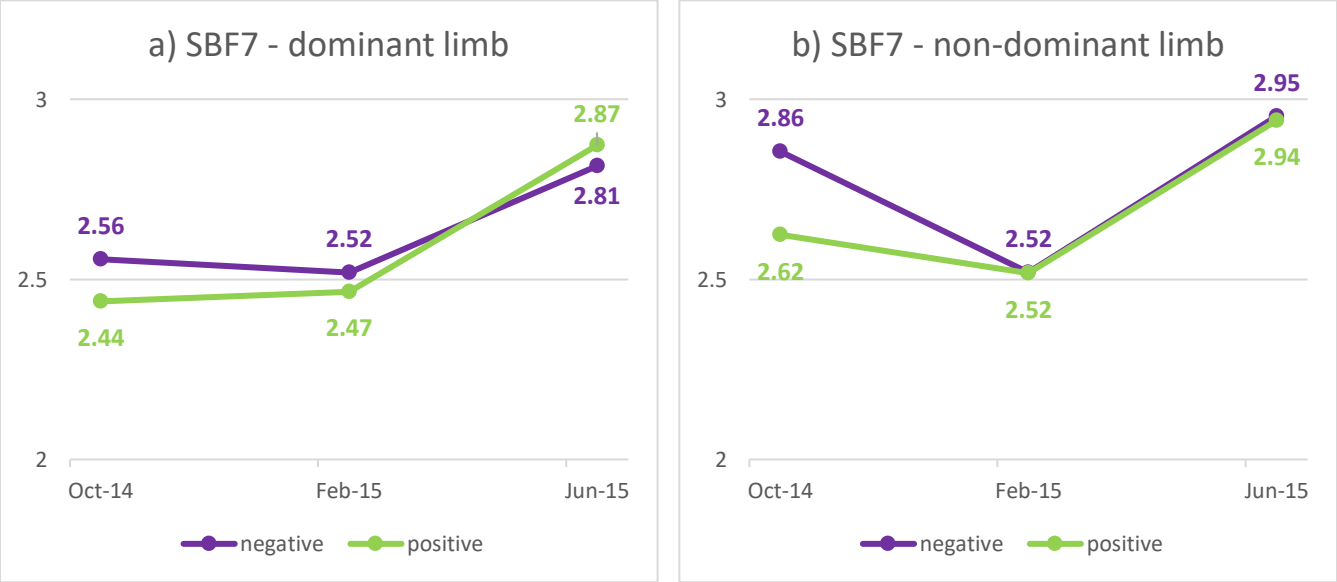


Figure 6.2.3.4: Mean BIS scores by infection group over three time-points at a) dominant limb, b) non-dominant limb.

6.2.3.5 Effect of PC on device scores

To determine the effect of PC on device scores, the groups were further divided by consumption of any PC and data collected during Feb-15 were excluded. This provided four groups for the before and after analysis: antigen positive cases who had taken any PC (n=24), antigen positive cases who had taken no PC (n = 10), antigen negative cases who had taken any PC (n = 27), and antigen negative cases who had taken no PC (n = 16). Unadjusted mean device scores by infection groups who did, or did not, take any PC can be seen in Figures 6.2.3.6 and 6.2.3.7 (lighter lines = participants who took PC, darker lines = participants who took no PC).

6.2.3.6 Effect of PC on tissue compressibility

At Jun-15, unadjusted cross-sectional comparison of LF-antigen positive cases who did, or did not, take PC showed that tissue compressibility was generally higher among cases who had taken any PC (Fig 6.2.3.6). This appeared to be clinically relevant at the dominant posterior thigh (9.5% higher among antigen positive cases who took PC, Fig 6.2.3.6-c), but none of the differences were statistically significant. The only measuring point where tissue compressibility was higher among the positive cases who had not taken any PC was at the non-dominant calf (1.1% p= 0.887, Figure 6.2.3.6-f).

There were also no significant differences in cross-sectional comparison of LF-antigen negative cases who had, or had not, taken PC but the between group pattern was reversed. Negative cases that had taken any PC generally returned lower (less compressible/stiffer) Indurometer scores at Jun-15 than those who had not taken PC with a slight exception for values at the non-dominant anterior thigh (consumed PC = 0.4% higher, p=0.901. Figure 6.2.3.6-b).

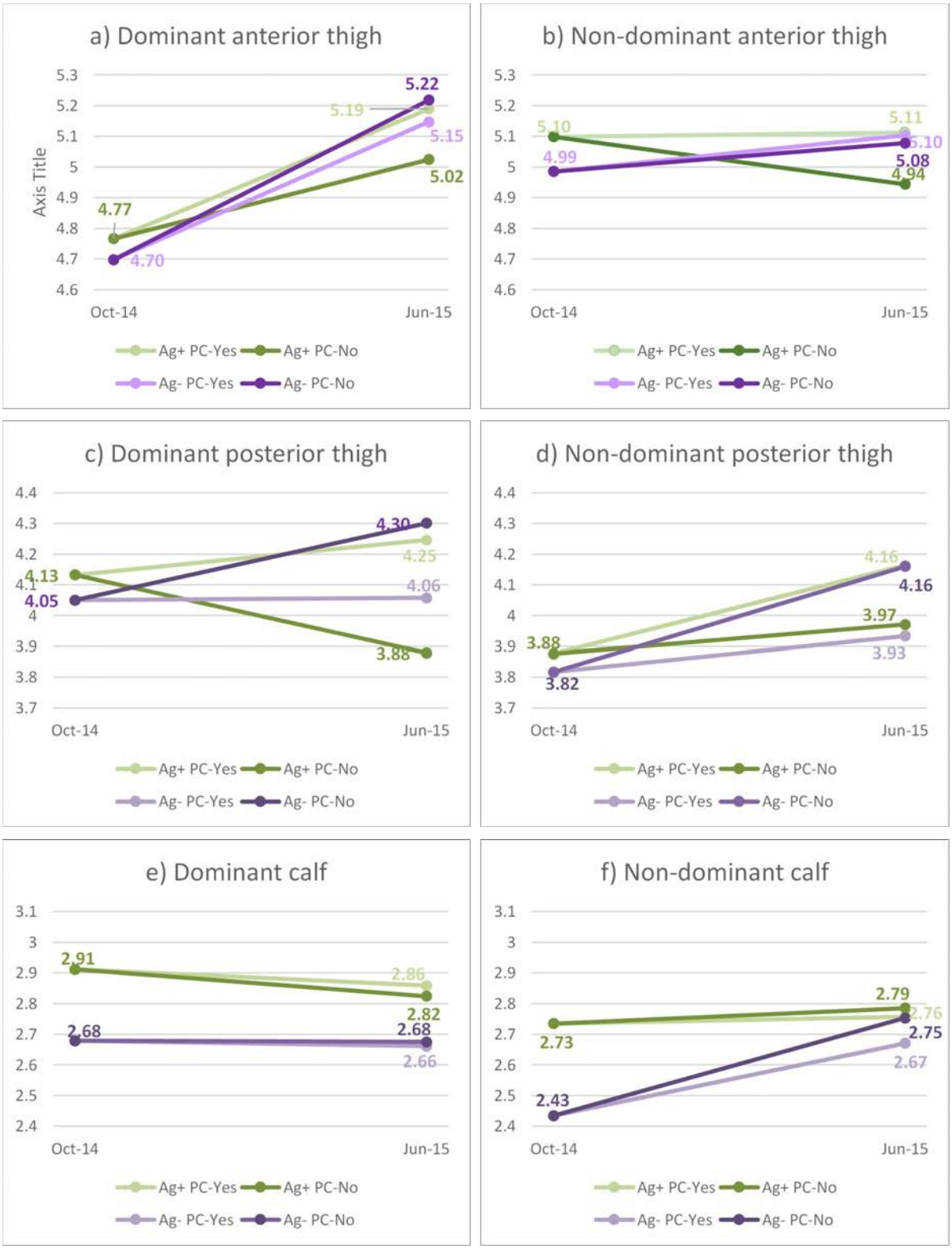


Figure 6.2.3.6: Unadjusted mean Indurometer scores by infection groups who did or did not take any PC at the a) dominant anterior thigh, b) non-dominant anterior thigh, at the c) dominant posterior thigh, d) non-dominant posterior thigh, e) dominant calf, f) non-dominant calf. Higher scores indicate more tissue compressibility (lighter lines = participants who took PC, darker lines = participants who took no PC)

6.2.3.7 Effect of PC on free fluid

Figure 6.2.3.7 shows the mean unadjusted BIS scores by infection group and PC consumption. Among the LF-antigen positive cases, cross-sectional analysis at Jun-15 of those who did, or did not, take PC delivered mixed results and none were significant. In the group who had taken any PC, free fluid was 3.6% lower in the dominant leg but 7.3% higher on the non-dominant side.

At Jun-15, antigen negative cases who had taken any PC had more free fluid in both legs than those who had not, and this difference was clinically relevant and statistically significant on the dominant side (10%, p=0.041, Figure 6.2.3.7-a), (non-dominant side 5.6%, p=0.352, Figure 6.2.3.7-b).

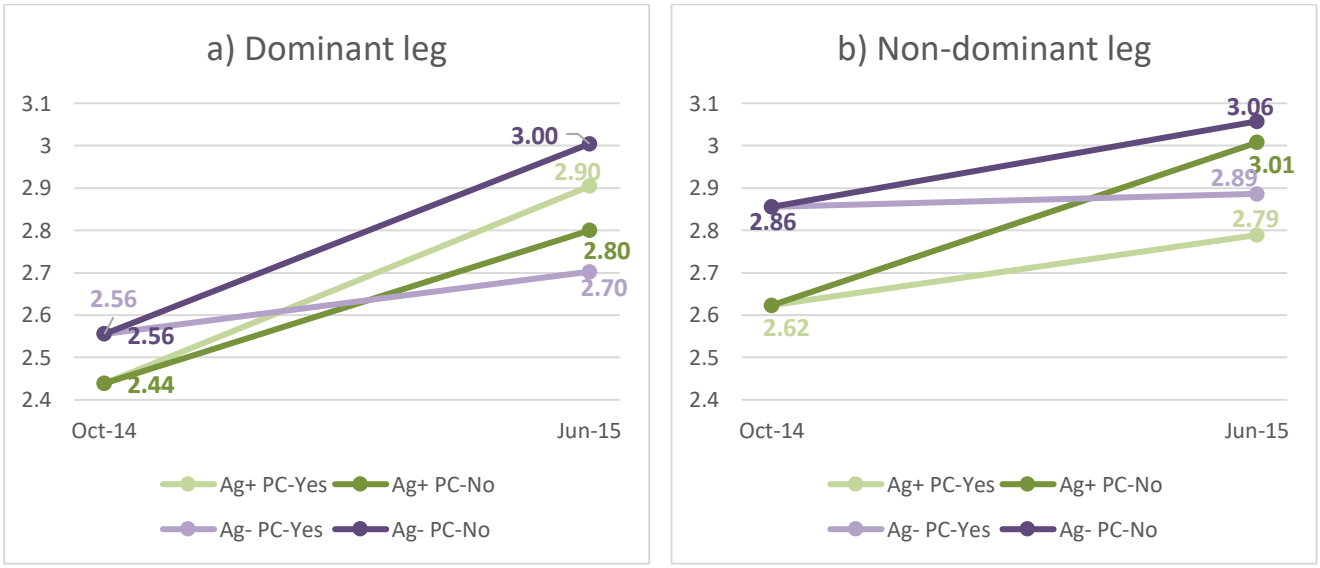


Figure 6.2.3.7: Mean BIS scores by infection group and PC consumption for the a) dominant limb and b) non-dominant limb. Higher scores indicate less free fluid (lighter lines = participants who took PC, darker lines = participants who took no PC).

6.2.3.8 Effect of moderating factors on tissue compressibility at the calf

Stepwise regression analysis at baseline (Oct-14) indicated that there was a clinically relevant and statistically significant association with increased tissue compressibility and LF-antigenemia at the non-dominant calf (11.6%, B = 0.30 (95%CI 0.069, 0.531), p=0.011). Table 6.2.3.8 shows all moderating factors for Indurometer measures at each calf. Accounting for age, gender, BMI-for-age, and hydration strengthened the association between LF-antigenemia and increased tissue compressibility at the non-dominant calf (B= 0.309 (95%CI 0.101, 0.517), p=0.004) and revealed a significant association between LF-antigenemia and increased tissue compressibility at the dominant calf (B = 0.246 (95%CI 0.022, 0.470) p=0.032). At Jun-15 follow up there were no significant relationships between LF-antigenemia or taking PC and tissue compressibility, which held true when other moderating factors were added to the model. There was a significant association between increased tissue compressibility and being female for all Indurometer measures at both timepoints and this was statistically significant at all measurement sites except the anterior thighs at Oct-14 and at the non-dominant anterior thigh at Jun-15 (data not shown in Table 6.2.3.8).

Table 6.2.3.8: Moderating factors associated with calf Indurometer scores at baseline and after PC. (Higher values = more tissue compressibility).

	Baseline October 2014		**Follow-up June 2015**	
	B (SE)		B (SE)	
Factor	Dominant	Non−dominant	Dominant	Non−dominant
Step 1 R^2 =	*0.034*	*0.066*	*0.029*	*0.006*
Antigen Positive	0.233 (0.128)	0.300 (0.116) *	0.182 (0.124)	0.069 (0.126)
PC=Yes	n/a	n/a	0.006 (0.131)	-0.060 (0.132)
Step 2 R^2 =	*0.284*	*0.276*	*0.308*	*0.299*
Antigen Positive	0.246 (0.113) *	0.309 (0.105) **	0.192 (0.110)	0.079 (0.111)
PC=Yes	n/a	n/a	0.047 (0.114)	-0.029 (0.115)
One year older	0.010 (0.017)	0.021 (0.016)	0.006 (0.017)	-0.011 (0.017)
Gender = Female	0.625 (0.188) ***	0.486 (0.110) ***	0.573 (0.117) ***	0.566 (0.117) ***
Underweight	-0.124 (0.141)	−0.080 (0.131)	0.032 (0.141)	0.108 (0.141)
Last drink >60 minutes	-0.159 (0.129)	−0.292 (0.120) *	-0.061 (0.113)	-0.186 (0.114)

** p< 0.05, ** p<0.01, *** p<0.00.*

6.2.3.9 Effect of moderating factors on free fluid

Stepwise regression analysis of baseline data (Oct-14) indicated that there was an association between increased free fluid and LF-antigenemia in the non-dominant leg (7.8%, p=0.062) which was statistically significant when other moderating factors were added to the model (B = -0.214 (95%CI -0.409, -0.020) p=0.031, table 6.2.3.9).

At Jun-15 there were no significant associations between free fluid loads and LF- antigenemia or with taking PC. At both time points, being female was significantly associated with a reduction in free fluid while being underweight was significantly associated with increased free fluid. There was an association between increased free fluid and age which was significant in both legs at Jun-15, but in only the dominant leg at Oct-14.

Table 6.2.3.9: Moderating factors associated with BIS scores at baseline and after PC. (Lower values = more free fluid).

	Baseline October 2014		**Follow-up June 2015**	
	B (SE)		B (SE)	
Factor	Dominant	Non–dominant	Dominant	Non–dominant
Step 1 R^2 =	*0.016*	*0.039*	*0.018*	
Antigen Positive	-0.117 (0.096)	-0.232 (0.123)	0.067 (0.124)	0.069 (0.126)
PC=Yes	n/a	n/a	-0.136 (0.130)	-0.060 (0.132)
Step 2 R^2 =	*0.307*	*0.426*	*0.339*	*0.421*
Antigen Positive	0.114 (0.083)	-0.214 (0.098) *	-0.044 (0.107)	-0.121 (0.104)
PC=Yes	n/a	n/a	-0.174 (0.111)	-0.032 (0.107)
One year older	-0.049 (0.012) ***	-0.058 (0.014)	-0.059 (0.016) **	-0.058 (0.016) ***
Gender = Female	0.229 (0.086) **	0.480 (0.101) ***	0.363 (0.114) **	0.500 (0.110) ***
Underweight	-0.246 (0.101) *	–0.339 (0.118) **	-0.344 (0.136) *	-0.331 (0.130) *
Last drink >60 minutes	0.110 (0.092)	0.124 (0.109)	0.009 (0.110)	0.180 (0.106)

* p< 0.05, ** p<0.01, *** p<0.00.

6.3 Summary discussion

MDA participation in this cohort was lower than required to break LF transmission (65%) and PC consumption among young antigen-positive males reduced between the 2013 and 2014 MDAs. Conversely, among young women, MDA participation increased which could imply that young women were more responsive to the MDA-related information provided at baseline.

On entry to the study, young people who were LF-antigen positive had higher tissue compressibility and more free fluid in the lower limbs compared to their antigen-negative peers. When other moderating factors were taken into account, there were statistically higher Indurometer scores at the calf and lower whole leg BIS scores (more fluid) on the non-dominant side. After consumption of PC the magnitude of the between-infection group differences had reduced, or even reversed direction and any remaining differences were not statistically significant.

A study over two years among young people with LF in India (including some with overt FRL) showed significant improvement in lymphatic pathology (by lymphoscintigraphy) after either 2 or 4 doses of PC (Kar et al., 2017). Follow-up of the Myanmar cohort after further MDA would help clarify if PC alone can completely reverse the covert tissue changes found using the Indurometer in the antigen-positive group.

From the analysis performed it cannot be determined if the significant, non-linear association with variation in device scores over time has a causal association with season. Hot weather on the day before measurement was reported to increase swelling in BCRL ($r= 0.27$, $p<0.001$) whereas a similar cohort of women without arm lymphoedema had no variation in ECF (Czerniec et al., 2016). Acute episodes of adeno-dermato-lymphangitis in Brugian

filariasis are more frequent in the rainy season in Southern India (Shenoy et al., 1998), but no studies have explored the effect of ambient temperature on tissue compressibility and extracellular fluid in people with LF.

Cross-sectional comparison of groups who had, or had not, taken any PC delivered some perplexing results. If LF was responsible for the between-infection group tissue changes found at baseline, and PC had reversed these changes, the untreated positive cases should be left with higher Indurometer scores (more tissue compressibility) than positive cases who took PC. But in some measures, the reverse was found and LF-positive cases who did not take PC generally had lower Indurometer scores than those who had taken PC. The net result was that baseline differences between antigen positive and negative participants had diminished by the follow up, after opportunity for treatment. However, the results for the different groups (antigen positive or negative, PC or no PC consumption) were inconsistent and further analysis accounting for covariates and time are required and are under way. Accounting for known moderating factors using linear regression did not resolve this discrepancy and modelling to account for variation due to time is required and is underway in collaboration with a statistician. This further analysis with multivariate modelling may also mitigate the problem of small group sizes in within-infection group comparisons of PC consumption, but some variation may also be due to other unmeasured factors such as level of daily activity or family income.

When moderating factors (age, gender, and BMI-for-age) were considered, the strong association between variation in device measures and gender reported at baseline (Chapter 5) were still present at follow-up. Females can be expected to have higher Indurometer and BIS scores than males (more compressible tissue, less free fluid) regardless of age or infection status. As young people grow, superficial tissue of the lower limbs become more

compressible (epifascial fat increases) and free fluid increases (increased muscle mass) but as may be expected with a data collection period of only nine months, year by year changes in this cohort were quite small. Being underweight was associated with reduced tissue compressibility and free fluid (less epifascial fat, less muscle mass) and should be considered during assessment of other physical parameters among similar populations.

6.4 Key points

- Covert tissue changes in antigen positive but asymptomatic young people can be detected using Indurometry and BIS.
- Passage of time between baseline and follow up measures contributed significantly to variation in device scores, and requires further analysis.
- After consumption of PC some covert tissue changes are reversed but this requires further analysis.
- Follow-up of participants after further MDA is required to determine the effect of PC on covert tissue changes.

Chapter 7: Interpretations and future directions

Presentations and posters on the research activities have been delivered at several meetings including the *Meeting of the Regional Program Review Group for Elimination of Lymphatic Filariasis and Soil Transmitted Helminths*, Bangkok, June 10, 2016. A full list of conference presentations can be viewed in Appendix F5. Further publications arising from the thesis will aim to assist program managers to implement early detection strategies and enhanced morbidity management protocols.

This Chapter will discuss how thesis findings have the potential to enhance and accelerate MMDP activities under the GPELF and Figure 7 shows the relevant research activities.

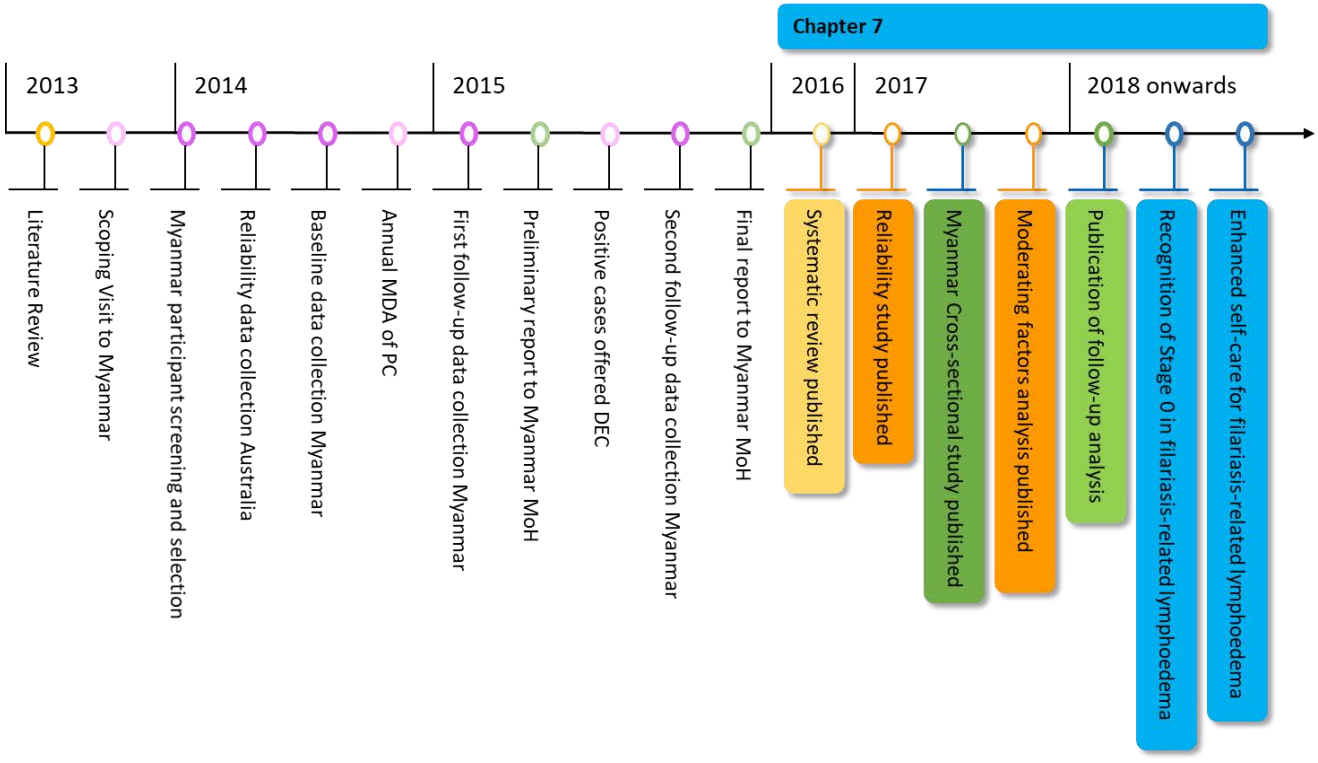


Figure 7: Research activities associated with discussion of the implications of thesis results and future recommendations for MMDP in the GPELF.

7.1 Thesis overview

Through exploring the risk factors, assessment techniques, and treatment recommendations for BCRL, it became clear that more can be done to identify and treat FRL at the earliest possible stage. This is not currently reflected in WHO guidelines for LF morbidity management which focus on preventing acute attacks and offer few recommendations to reverse mild disease. The primary objective of this thesis was to determine if covert connective tissue changes associated with lymphatic dysfunction can be detected in asymptomatic, LF positive cases. Secondary outcomes were to identify field-friendly devices to quantify these changes and assess the effect of PC consumption.

Lymphoedema has affected the lives of people for millennia, and today is still a major cause of disability worldwide with LF causing most of the global burden. Progression to advanced stages is accompanied by increasing deformity and disability but individual risk-factors that promote disease progression are not well understood. It is possible to interrupt LF transmission with existing technology and there need be no new cases in the foreseeable future. However, both before and after transmission has stopped, existing and future cases of FRL will need lifelong MMDP services and this will require ongoing government and donor support well beyond 2020.

The disparity in resources between BCRL and FRL settings should not be a barrier to transferring reliable and effective protocols for early detection and intervention in lymphoedema to LF populations.

7.2 Tissue compressibility and free fluid in the lower limbs

Usually, FRL appears distally and progresses proximally, so detectable tissue changes may occur earlier at the calf than at the thigh. The relatively thin layer of skin and tissue over the muscle of the calf may also render early tissue changes more evident here than in fattier parts of the leg. Accordingly, the association between LF antigenemia, and increased tissue compressibility was statistically significant at mid-calf, and large enough on the non-dominant side to also be clinically relevant. BIS scores also show more free fluid in the non-dominant side in the positive cases and this early appearance of lymphatic dysfunction in the non-dominant leg is consistent with reports on BCRL which show an increased risk of arm lymphoedema if the operated side is also the non-dominant arm. A tendency for fluid to accumulate more readily on the non-dominant side could be the result of differences in muscular activity which naturally promotes lymph flow. If daily muscle use is less forceful or less frequent on the non-dominant side, lymph flow may be lower on that side too.

The Indurometer and SBF7 were both able to detect small but clinically relevant variation in tissue composition associated with age, gender, LF-antigenemia, and habitual patterns of muscle use. Fluctuating influences such as the female hormonal cycle and systemic hydration were also frequently detected. This suggests that both devices may be valuable in detecting small changes in connective tissue pathology in FRL and evidence to establish their reliability in manifest lower limb lymphoedema is now needed.

Primary lymphoedema in young people is another under-researched area in which the devices and moderating factors investigated may be important. The population data and range of variation in device measures already published will be useful for clinical trials or studies in the lower limb of young people when the contralateral leg is not suitable for

comparison, and may be used to estimate expected values and cut-off points in future research on young populations at risk of lower limb lymphoedema.

7.2.1 Indurometer

Indurometry has demonstrated excellent reliability with a single operator (Chapter 4) and low variability among multiple operators, however Vanderstelt, Pallotta et al. (2015) noted the greatest inter-operator variability when assessing stage 1 BCRL. At this early fluid rich stage, tissue changes are still in a state of flux and the pitting nature of the subcutaneous tissue means that indentation can affect repeated readings at the same location, a frequent issue with the mechanical Tonometer. The Indurometer has overcome this to some extent by capturing the measure within microseconds of the correct load being applied. As the device is removed from the skin immediately, persistent pitting should not remain at the location after the measure, but this can still be an issue when the oedema is very soft, especially at the forearm. Like the mid-calf, the forearm has a naturally thin fat layer which may make it easier to detect small changes in tissue stiffness. In covert and early lymphoedema, thixotropic changes in the extracellular matrix flux and wane along an ill-defined continuum before the more rigid fibrotic changes set in during stage 2 when tissue fibrosis will be overt and less susceptible to fluctuation. The large and significant variation in Indurometry at the calf at baseline, and higher rate of variability at the forearm during stage 1 BCRL, point to the mid-point of the distal arm and leg as appropriate assessment site when looking for small connective tissue changes in at-risk cases. In early stage pitting oedema, allowing time for any residual indentation to resolve between repeated measures is recommended.

Based on the reliability and agreement found between devices, this study has helped to establish the Indurometer as an acceptable replacement for the mechanical tonometer and a potentially useful device in FRL. With an inbuilt sensor which rejects any measure if the

device is applied erratically, correct technique can be learned with minimal training, requires no ongoing maintenance or consumables, and can be calibrated regularly by the operator. It is field-friendly and suitable for both clinical and research use.

7.2.2 Bio-impedance spectroscopy

Bio-impedance spectroscopy has become an important tool in both clinical practice and research on BCRL and is a non-invasive method to objectively measure extracellular fluid loads in the connective tissue. In keeping with multiple previous reports on BIS, the SBF7 used in this study showed excellent reliability in the lower limbs of the young Australian and Myanmar cohorts. These findings widen the demographic in which BIS can be used to detect small fluctuations in ECF load in the lower limbs and should be considered in research in LF populations. The necessity for multiple, relatively expensive, single-use self-adhesive electrodes for each measure makes it less practical than the Indurometer in a rural-health setting.

7.2.3 Moderating factors in device measures

There were universal variations in Indurometer and BIS scores associated with age and gender as well as distinct trends in limb dominance, BMI-for-age, systemic hydration, and the female menstrual cycle. A year by year increase in tissue compressibility (more epifascial fat) and free fluid (more muscle mass) was consistent with normal linear growth and among females, tissue compressibility was higher (higher fat to muscle ratio) and free fluid was lower (less muscle mass) than males. In the longitudinal analysis on the Myanmar data, these strong influences of age and gender were maintained over time regardless of LF status or PC consumption.

The strong association between BMI and BCRL demanded that this variable be considered, but normal BMI for adults in Asian countries can be 3kg/m^2 less than Western standards (WHO, 2004) and adult values are not relevant for children (Duncan, 2009). Using readily available online resources from the CDC and WHO, two methods to determine appropriate BMI-for-age were used in this study. The CDC charts were more aligned to the young Australians and classified twice as many young Myanmar people as underweight (27 vs 13) than the WHO charts and criteria which were more aligned to the Myanmar cohort. Using either scale, with all devices, being underweight was associated with reduced tissue compressibility and free fluid (less epifascial fat, less muscle mass) for some measures. On a geographic scale, the pattern of tissue compressibility noted between the PNG (least compressible), Myanmar, and Australian cohorts (most compressible, Chapter 4 section 4.4.1) suggests an association between increased epifascial fat and economic development.

Being in the second half of the menstrual cycle was frequently associated with a larger, softer limb and more free fluid. As the average age of breast cancer detection decreases and survival rates increase, more women will be at risk of BCRL at a younger age. In these populations, age and regular hormonal fluctuations should be considered and accounted for when assessing BCRL. Hydration as represented by the time since a last drink, contributed to variance in all devices and wherever such relationships were found, not having a drink within the previous hour was associated with increased tissue stiffness. These factors could be controlled by administering a standardised drink prior to measurements, and timing repeated measures on young women at the same time of the monthly cycle.

7.3 Defining Stage 0

Following on from the pilot study in PNG (Gordon et al., 2011), the research activities of this thesis have contributed empirical evidence to support the presence of covert but measurable increases in tissue compressibility and free fluid associated with LF antigenemia. The advance over the PNG study was the addition of the electro-mechanical tonometry devices and inclusion of other moderating factors to confirm an independent effect of infection. The presence and direction of clinically relevant connective tissue changes in the antigen positive cases found in Myanmar supports the hypothesis that LF can induce covert but measurable changes in the subcutaneous compartment. Other evidence for covert tissue pathology in asymptomatic cases has previously relied on expensive or invasive, hospital-based procedures such as lymphoscintigraphy (nuclear imaging) or tissue biopsy (Kar et al., 2017; Shenoy et al., 2008; Wilson et al., 2004). The Indurometer and BIS offer novel, non-invasive devices for objective measurement of covert tissue changes in FRL. Follow-up on the Myanmar cohort, with consideration for PC consumption, may provide some insight into individual variation among antigen-positive persons with covert tissue changes who do or do not progress to more overt disease.

7.3.1 Why size matters

Clinical lymphologists and researchers use a range of criteria to diagnose, stage, and measure treatment outcomes in lymphoedema. The magnitude of change required to be clinically relevant is poorly evidenced and there is no universally accepted parameter or cut-off point to determine when the label of lymphoedema should be applied. Therefore, professional guidelines are developed from a contextual synthesis of research outputs, clinical observation and expert opinion. With this in mind, and in keeping with accepted guidelines, a 10% difference in tissue compressibility was used to represent the magnitude of variation

required to be clinically relevant between-infection groups at baseline. At follow up, this was reduced to 5% in order to detect the smallest, plausible effect of the intervention within accepted guidelines. The 5% criteria may increase the risk of variability due to normal connective tissue flux rather than pathological change, therefore the 10% criteria, already commonly used in BCRL, is a more credible figure. Free fluid in the extracellular compartment also fluctuates depending on multiple factors but normal homeostasis will keep this quite low, therefore the cut-off point of 3% for BIS scores was maintained for all analyses.

Whether a 10% change in Indurometry scores at mid-calf or a 3% increase in BIS measures is enough to classify the connective tissue changes as pathologic is not known. Further investigation of both devices in covert and manifest FRL may clarify the percentage change needed to determine a meaningful magnitude of variation in tissue pathology.

7.3.2 The effect of PC on covert changes in Myanmar

The clinically relevant association between LF-antigenemia and variation in Indurometer and BIS scores at baseline could not be found after 66% of the cohort had consumed PC. Variances in Indurometer scores at the dominant calf were still large enough to be considered clinically relevant, but the large and significant difference in non-dominant calf scores seen at baseline was no longer evident. When infection groups were stratified by PC consumption, some groups were too small to deliver any statistical relevance (eg LF-antigen positive cases who had taken no PC, n = 10) but by every comparison, the magnitude of the association between device scores and LF-antigenemia reduced between October 2014 and June 2015. Observed changes in the antigen-positive group either reversed or remained stable compared to the antigen-negative participants. However, the results for the different groups (antigen-positive or negative, PC or no PC consumption) were inconsistent and further analysis accounting for covariates and time are required and are under way.

Other reports on complete reversal of early or covert FRL have been reported where the whole cohort consumed multiple, predefined doses of PC medication, sometimes in conjunction with antibiotics and followed for up to two years (Kar et al., 2017; Shenoy et al., 2009). It is not known if the required 65% MDA coverage in real-life settings will also achieve this reversing effect. Even if 65% coverage is adequate, in the Myanmar cohort MDA participation was much lower than this. Other townships in Central Myanmar may also have less than adequate MDA coverage as despite commencing the LF elimination program in 2001, few regions have had more than six rounds of MDA to 2015 (Dickson et al., 2015). The convenience sample in this study can't be extrapolated to larger populations; however, among everyone screened for LF during recruitment into the longitudinal study, the overall rate of infection (detected by ICT) was very high (close to 20%) and there was poor uptake of the MDA especially among the positive cases (2013 MDA = 34%. 2014 – 59.5%). Hence a high number of people are at risk for cumulative effects of LF exposure and even if MDA alone could reverse covert or early stage disease, some positive cases will progress to overt FRL. A longer follow-up after further PC would be valuable to answer this question. In the meantime, relying on MDA alone to reverse early FRL will potentially allow more cases to progress to advanced disease in many endemic countries.

7.4 Study limitations

The sample was large enough to provide statistical significance for most analyses, but convenience sampling meant the data is not truly representative of each population and not directly transferrable to other settings. The principal investigator, as a single operator, could not be blinded to any of the parameters measured (except the baseline antigen testing and initial measurement), but the use of local Myanmar research assistants increased the level of accuracy in data recording and reduced the risk of bias by visually confirming each measure.

The standard statistical tests used are validated for large populations with few moderating variables (Leek, 2007), but lymphoedema rarely offers such an ideal scenario. Fluctuating tissue composition and unknown risk-factors are difficult to measure and the results of this analysis should be viewed cautiously. The large number of tests conducted create a possibility that the single statistically significant difference found at baseline occurred by chance. This may have been mitigated to some extent by the results of the Tonometer which also showed softening of the non-dominant calf among the positive cohort, as well as and the increase fluid found by BIS (Figure 5.2.3.2). Until the data are analysed more rigorously, the inclusion of descriptive outputs and consideration of effect size have been used to offer some insight into any covert tissue changes that might be occurring within this LF endemic cohort.

The original study design was based on an expectation that >65% of the study population would participate in the 2014 MDA. Had the actual, low level of coverage been anticipated, more effort could have been made to ensure that antigen positive participants took the MDA. However, as the intention was to follow a population before and after real-life MDA conditions (in contrast to other studies that have provided PC to all participants), the unexpected results in MDA coverage have underscored the danger in extrapolating research data to real-life scenarios.

7.4.1 Variation associated with time.

At first thought to be a drawback, the need to collect a second set of follow-up data allowed for the discovery of the effect of the time on device scores. Without the third data set, before and after PC comparison would have given a very different result. This variation could be interpreted as a seasonal influence, but a causal effect of season cannot be confirmed with the current descriptive analysis. The complex modelling required to tease out the effect of season is beyond the current capacity of the author and will be performed in collaboration with a biostatistician.

The possible effect of weather is worth considering as the fatty tissue of the epifascial compartment compensates for body temperature by either losing or trapping heat and plays an important role in holding or releasing water to maintain fluid homeostasis. Myanmar has three main seasons; a warm wet season, followed by a cooler dry season, and a hot wet season. Mandalay Region, in Central Myanmar is known as the dry zone and classified as hot and semi-arid by the Köppen-Geiger scale (Kottek, Grieser, Beck, Rudolf, & Rubel, 2006). Directional variation at each time point coincides with the warm-wet, cool-dry, and hot-wet seasons respectively. If interpreted within the context of known factors associated with fluid homeostasis in humans, the increase in tissue compressibility and reduction in free fluid in warmer more humid weather may be due to water being held within the connective tissue matrix as a buffer against dehydration. Additionally, in the hot humid season more participants reported having a recent drink highlighting the difficulty in remaining well hydrated in these weather conditions.

7.5 Standing on one leg

Referred to as the 'twin pillars' of the GPELF, MDA and MMDP are rarely applied equally, with MDA preceding MMDP by many years in most countries. This sequential approach to GPELF activities has seen services for existing cases take a back seat and can be attributed, in part, to the complexity involved in managing lymphoedema and the lack of operational research on country specific requirements and expected benefits (Kumari et al., 2012; Narahari et al., 2017).

Despite the dramatic images of advanced FRL typically used to attract donor funding for MDA activities, MDA offers no improvement for existing cases, and the lag in time before MMDP activities are commenced allows mild and moderate cases to progress, thereby increasing the number of advanced cases requiring future support services. Once MMDP activities are

commenced, sequential strategies such as mapping of existing cases may also lead to unintended consequences such as increased stigmatisation, and identifying cases without offering education in self-care at the same time could be considered unethical. There is evidence that implementing MMDP activities before MDA can increase MDA participation (Cantey, Rout, Rao, Williamson, & Fox, 2010) and applying both pillars simultaneously may produce other synergistic benefits.

Early detection of covert tissue changes and implementation of preventive strategies may be the ideal to prevent new overt cases, but without specific strategies and resources to identify covert and early FRL National LF Program Managers will struggle to find support for preventive interventions. One way to address this may be to provide education in self-care to whole at-risk communities, not just the overt cases. This would provide those who already have covert disease the opportunity to prevent their own progression.

For FRL management activities to be viably scaled up in all LF endemic areas, inexpensive, field-friendly devices which can reliably detect subtle changes in FRL status are needed. The ability of both the Indurometer and BIS to detect subclinical changes associated with lymphatic dysfunction indicate that they may provide the much-needed empirical evidence to support enhanced morbidity management under the GPELF. Both devices will be valuable in operational and clinical research on LF morbidity and the Indurometer also offers an inexpensive, field-friendly, clinical measure suitable for use at the health centre or community level.

7.6 Key points

- Indurometry and BIS detected subclinical changes associated with lymphatic dysfunction in LF-positive cases.
- This evidence establishes the need for formal recognition of stage 0 in FRL assessment criteria.
- Morbidity management for FRL will need to continue after LF transmission is stopped and will require ongoing donor support beyond 2020.
- Preventive recommendations under the GPELF will assist program managers to develop appropriate strategies to identify and address at-risk cases.
- WHO guidelines for MMDP do not provide strategies to reverse covert or early stage disease.
- Community level education in self-care in at-risk populations may prevent progression of covert FRL.
- Indurometry and BIS offer novel, non-invasive measures to assess FRL.
- Establishing an appropriate surveillance period for MMDP activities after PC has ceased will help to identify at-risk cases and early stage FRL.

7.7 Final Conclusions

This research project identified gaps in the literature concerning FRL, particularly compared to the more researched BCRL. However, assessment techniques and prevention strategies currently used in BCRL could be employed in FRL settings. Indurometry and BIS were found reliable in the Myanmar cohort and able to detect subclinical changes in LF-positive cases. These findings contribute to current understanding of progression to overt FRL and highlight the need for criteria to identify stage 0. Taken together, this new approach and reliable technologies can be used to support the GPELF in developing enhanced morbidity management strategies to help the much neglected people with FRL worldwide.

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Appendices

Appendix A: Memorandum of Understanding and preliminary reports to Myanmar MOHS

Appendix A1: Memorandum of understanding between JCU and Myanmar MOHS

Appendix A2: Preliminary report 1

Appendix A3: Preliminary report 2

Appendix A1: Memorandum of understanding between JCU and Myanmar MOHS

MEMORANDUM OF UNDERSTANDING

between

JAMES COOK UNIVERSITY
ABN 46 253 211 955

and

REPUBLIC OF THE UNION OF MYANMAR
MINISTRY OF HEALTH (MOH)

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Appendix A2: Preliminary report 1

A preliminary report on the research project entitled

Improved Strategies for Prevention of Life Long Disability from Lymphatic Filariasis in Myanmar

Baseline data collection October 2014

The support and co-operation of all Local Authorities and Government Departments is gratefully acknowledged.

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10 November 2014

Screening and Participant selection

Immuno-Chromatographic Test (ICT) cards were used to screen all volunteers for evidence of infection with Lymphatic Filariasis (LF) a mosquito borne disease which causes elephantiasis and hydrocele. Selected positive and negative cases were then invited to have physical measures taken of their legs and provide a 10ml blood sample.

All tests were carried out in Thar Le Swart Quarter Administration Office on young people aged 10 – 21 years residing in Amarapura Township.

315 tests were performed, 7 tests failed to provide a clear result and 61 positive results were recorded, a rate of 61/308 = 19.81% overall. The rate of infection varied between Quarters. Most people were residents of Sar Kyin Wa (52.38%) where the infection rate was 12.12%. Double this rate was found among the residents of Thar Le Swart where the rate was 25.81%. Higher rates of infection were found in other villages but these samples sizes would be too small individually to draw reliable conclusions about infection rates in those locations.

In the study sample most participants were female (56.51%) and aged 10 - 17 years (71.11%).

The following table indicates the positive and negative rate by gender, age, and residential location.

N (%)	Positive	Negative	Failed tests	Total
Male	27 (19.71)	105 (76.64)	5 (3.65)	137 (43.49)
Female	34 (19.10)	142 (79.78)	2 (1.12)	178 (56.51)
10-17 year olds	40 (17.86)	179 (79.91)	5 (2.23)	224 (71.11)
18 – 21 year olds	21 (23.08)	68 (74.73)	2 (2.19)	91 (28.89)
Thar Le Swart	24 (25.81)	69 (74.19)	0	93 (29.52)
Sar Kyin Wa	20 (12.12)	139 (84 24)	6 (3.64)	165 (52.38)
Other village*	15 (31.91)	31 (65.96)	1 (2.13)	47 (14.92)
Unknown residence	2 (20.0)	8 (80.0)	0	10 (3.17)
Total	**61**	**247**	**7**	**315**

* *Combined data from Kyaung Shar, Kyaung Shime, Mayan Chan, Paung Taw, Shwe Gae, Tar Mynint Naing, Sin Gar, Lat Owe Myint Naing, Sar Kyar and Wae Myin Taing,*

57 positive young people were matched for age and gender with 57 negative cases and these 114 people were selected to have physical measurements of their legs and to provide a venous blood sample.

Baseline Measurements

114 participants (57 positive and 57 negative) were selected for baseline measures in the longitudinal study, of these, ten participants could not be found.

All participants (104) were weighed and their height was recorded along with specific limb and skin measurements to determine if subclinical lymphatic disorders can be detected by these physical measures. Venous blood samples were collected and will be analysed to identify biochemical indicators of early lymphatic disorder.

Participants in these measures we asked if they had consumed the deworming drugs during the 2013 MDA. Less than 40% reported they had taken the drugs and of these 17 had tested positive on ICT indicating a consumption rate of 41.46% among participants who are infected with LF. Of the remaining participants who reported they did not consume or did not receive the deworming medication in 2013, 29.18% tested positive, however these small samples should not be taken as representative of the wider population

The following table indicates rate of consumption of MDA in 2013 by gender, age and location.

	N	N=Consumed MDA 2013	% Consumed MDA 2013
Male	46	30	65%
Female	58	11	19%
10-17 year olds	70	22	31%
18 – 21 year olds	34	19	56%
Thar Le Swart	41	11	26.83%
Sar Kyin Wa	54	24	44.44%
Other village	9	6	66.67%
Total	104 (31+ve)	41 (17+ve)	39.42%

Whilst these groupings generate samples sizes too small to offer meaningful statistics MDA consumption rates they do indicate that more needs to be done to engage the population generally during the MDA in this area, especially among young girls and residents of Thar Le Swart.

A list of people who tested positive for LF and stated that they did not consume MDA drugs in 2013 has been provided to the VBDC in Mandalay. Dr San San Win of the WHO has recommended that these persons be given deworming medication. They should also be targeted to ensure participation in the 2014 MDA.

Future Surveys

The 104 persons measured during October 2014, will be invited to have the same measurements taken during February 2015 (after they have consumed the 2014 MDA)

A morbidity prevalence survey is also planned for the wider Mandalay Region incorporating five Townships and 300 households (approx. 1500 people). This is planned to be conducted during January and March 2015.

Appendix A3: Preliminary report 2

A preliminary report on the research project entitled

Improved Strategies for Prevention of Life Long Disability from Lymphatic Filariasis in Myanmar

Follow up data collection February 2015

The support and co-operation of all Local Authorities and Government Departments is gratefully acknowledged.

In particular

Regional Health Director Dr Than Win
Amarapura Township Medical Officer Dr Aung Naign Oo
VBDC Team Leader Dr Tint Wai Tun
PHL Manager Dr Yi Yi
Amarapura Administrator Mr Nay Myat Thu

and

Quarter Leaders
Mr Ko Tint Lwin
Mr Myo Chit
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19 February 2015

Participant recall and follow up measures

All follow up measurements were carried out in the Quarter Administration Office on young people aged 10 – 21 years residing in Amarapura Township who participated in baseline measurement during October 2014.

Of the 104 participants measured in October 2014, 9 were lost to follow up and 95 were re-measured in February 2015 (91.35%). In the follow up population most participants were female (57.89%) and aged 10 - 17 years (68.42%).

Of the 95 returning participants, 40 positive and 40 negative cases are age and gender matched pairs. 15 of the participants measured do not have an age and gender matched pair.

Five participants who tested as negative for LF in 2014, retested as positive by ICT in 2015. And one person who had tested positive in 2014, retested as negative by ICT in 2015. These positive and negative results will be confirmed when filter paper blood samples are analysed during 2015.

The following table indicates the positive and negative rate among follow up participants by gender, age, and residential location.

N (%)	Positive	Negative	Total
Male	21 (52.50)	19(47.50)	40 (42.11)
Female	26 (47.27)	29 (52.73)	55 (57.89)
10-17 year olds	31 (44.29)	34 (55.71)	65 (68.42)
18 – 21 year olds	16 (53.33)	14 (46.67)	30 (31.58)
Thar Le Swart	16 (66.67)	8 (33.33)	24 (25.26)
Sar Kyin Wa	21 (36.21)	37 (63.79)	58 (61.05)
Other village*	10 (76.92)	3 (23.08)	13 (13.68)
Total	**47**	**48**	**95**

* *Combined data from Kyaung Shar, Kyaung Shime, Mayan Chan, Sin Gar,*

Follow up measurements

All participants (95) were weighed and their height was recorded along with specific limb and skin measurements to determine if subclinical lymphatic disorders can be detected by these physical measures. Venous blood samples were collected on 94 participants and will be analysed to identify biochemical indicators of early lymphatic disorder and the effect of MDA consumption on these biochemical markers.

MDA Consumption

Participants were asked if they had consumed the deworming drugs during the 2014 MDA. Thirty – nine (41.05%) reported they had taken the drugs and of these 23 had tested positive on ICT indicating a consumption rate of 48.94% among participants who are infected with LF. This is an increase on figures found in the October data (41.46% of infected participants). Of the remaining

participants who reported they did not consume or did not receive the deworming medication in 2014, 42.86% tested positive.

These selected follow up participants should not be taken as representative of the wider population. Rather it should be noted that 22 participants that did not consume the MDA in 2013 did consume the deworming drugs in 2014 and 25 participants who stated that they had consumed the MDA in 2013 did not consume the deworming drugs in 2014.

Importantly for the power calculation in this study 22 people who tested positive for LF in 2014 did not consume the MDA, leaving a sample of only 21 positive cases whose measurements and blood samples can be used to determine the effects of the MDA on early lymphatic changes. Of these it appears that 3 were over 21 years of age at baseline and will be excluded from the final analysis.

The following table indicates rate of consumption of MDA in 2014 by gender, age and location.

	N 2014	**N(%) Consumed MDA 2013**	**N 2015**	**N(%) Consumed MDA 2014**
Male	46	30 (65.22)	40	15 (37.50)
Female	58	11 (18.97)	55	24 (43.67)
10-17 year olds	70	22 (31.43)	65	27 (41.54)
18 – 21 year olds	34	19 (55.88)	30	12 (40.00)
Thar Le Swart	41	11 (26.83)	24	13 (54.17)
Sar Kyin Wa	54	24 (44.44)	58*	21 (36.21)
Other village	9	6 (66.67)	13	5 (38.46)
Total	104 (48+ve)	41 (17+ve) 39.42%	95 (47+ve)	39 (23+ve) 41.05%

* Incorrect or missing residential village data was clarified during follow up data collection

Appendix B: Ethics approvals

- *Appendix B1: JCU Human Research Ethics Committee, Myanmar cohort approval*
- *Appendix B2: Myanmar MOHS, Myanmar cohort approval*
- *Appendix B3: JCU Human Research Ethics Committee, Australian cohort approval*

Appendix B1: JCU Human Research Ethics Committee, Myanmar cohort approval

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Appendix B2: Myanmar MOHS, Myanmar cohort approval

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Appendix B3: JCU Human Research Ethics Committee, Australian cohort approval

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Appendix C: Participant Information Sheets and Consent Forms

Appendix C1: Participant Information Sheet and Consent Form – Myanmar, English

Appendix C2: Participant Information Sheet and Consent Form – Adult, Myanmar language

Appendix C3: Participant Information Sheet and Consent Form – Child, Myanmar language

Appendix C4: Participant Information Sheet and Consent Form – Adult, Australia

Appendix C5: Participant Information Sheet and Consent Form – Child, Australia

Appendix C1: Participant Information Sheet and Consent Form – Myanmar, English

INFORMATION SHEET "Understanding lymphatic filariasis in children and adolescents"

You are invited to take part in a research project to help understand the effect of lymphatic filariasis in children and adolescents. By understanding the changes that happen we will be able to plan better ways to stop the development of a big leg or hydrocele. The study is being conducted by Jan Douglass, supervised by Associate Professor Susan Gordon and Associate Professor Patricia Graves and this research will contribute to Jan Douglass' Doctor of Philosophy degree at James Cook University.

This study will be done as part of the Mass Drug Administration (MDA) project in your district. Members of the Myanmar MDA project team will assist with all measurements.

If you consent for your son or daughter to be involved in the study, you will be asked some questions to make sure your child has not had any recent illness and is fit to be included in this study. It would be best if you were able to be present for all the measures. Your childs' finger will be pricked and a drop of blood will be placed on a piece of card. This card tests to see if your child has lymphatic filariasis. The results of this test will be recorded as part of this study and will be compared to the other measurements which are part of this study. Your child will then be weighed and their height will be measured. They will then lie down with their legs uncovered – it will be best to wear shorts or bathers so the legs can be measured. Four types of measurements will be taken. All of them are safe, standard clinical tests which will not cause any pain or discomfort.

1. The size of the legs will be measured. The midpoint of your childs' legs and thighs will be marked with a pen and then measurements will be taken at these marks with a tape measure.
2. The softness of the back and front of the thigh, and the back of the calf will be measured. Your child will be asked to lay on their back for the front of thigh measurement and on their tummy for the back of thigh and calf measures. A small unit called an indurometer will be placed on the skin at the midpoint of the thigh and calf. The unit has a small plunger with a 200gram weight attached. The device measures how far the plunger moves into the skin and how much resistance the skin provides when the 200gram weight is applied to the skin.
3. The amount of fluid in the thigh and leg will be measured. This is done electrically using a Bioelectrical Impedance device. A small skin electrode will be placed on each of your child's arms, ankles and knees. The risk of skin irritation from the electrodes is minimal. A low level electrical current will be passed via the electrodes and will provide a measure of the amount of fluid in the thigh and in the calf. The only sensation your child will feel when the device is in use is the pressure of the sticky electrode on their skin. In preparation for measurement all jewellery must be removed. At all times the device will be used within manufacturer standards.
4. An ultrasound machine will be used to see if there are any worm nests in the lymph nodes of the groin. Some water based gel will be placed over the lymph nodes in the groin. The ultrasound will then be placed on the skin – if any worms are identified an image of the worms will be recorded.

The measurements should take approximately 45 minutes of your time.

Taking part in this study is completely voluntary and you can stop your child taking part in the study at any time without explanation or prejudice.

It is not anticipated that your child will experience any discomfort or harm from participation in this study, nor will they receive any benefit or payment.

The information from this study will be used in research publications, presentations and reports. Your child will not be identified in any way in these publications.

If you have any questions about the study, please contact

Principal Investigator: Jan Douglass
School of Public Health Tropical Medicine and Rehabilitation Science
James Cook University
Phone: 0011 61 419 848 589
Myanmar contact number: TBC
Email: Jan.douglass@my.jcu.edu.au

Supervisor: Associate Professor Sue Gordon
School of Public Health Tropical Medicine and Rehabilitation Science
James Cook University
Phone: 0011 61 7 407885699
Myanmar contact number: TBC
Email: susan.gordon2@jcu.edu.au

If you have any concerns regarding the ethical conduct of the study, please contact:
Human Ethics, Research Office
James Cook University, Townsville, Qld, 4811
Phone: (07) 4781 5011 (ethics@jcu.edu.au)

INFORMED CONSENT FORM

PRINCIPAL INVESTIGATOR	**Jan Douglass**
SUPERVISORS	**Associate Professor Susan Gordon & Associate Professor Patricia Graves**
PROJECT TITLE:	**"Understanding lymphatic filariasis in children and adolescents"**
SCHOOL	**School of Public Health Tropical Medicine and Rehabilitation Science**

I understand the aim of this research study is to understand the effect of lymphatic filariasis on children and adolescents. I consent to my child participating in this project, the details of which have been explained to me, and I have been provided with a written information sheet to keep.

I understand that I will be asked some questions regarding my childs' health before the study starts. My childs' participation will involve measurement of the size of my childs' legs using a tape measure, measurement of the softness of the thigh and calf using an indurometer, measurement of the amount of fluid in the legs using a bioimpedance machine and identification of worm nests in the groin using an ultrasound machine. I agree that the researcher may use the results as described in the information sheet.

I acknowledge that:

- taking part in this study is voluntary and I am aware that I can stop my child taking part in it at any time without explanation or prejudice and to withdraw any unprocessed data provided;

- that any information I give will be kept strictly confidential and that no names will be used to identify my child with this study without my approval;

(Please tick to indicate consent)

I consent to my childs' finger being pricked and a sample of blood being placed on cardboard for testing		**Yes**		**No**
I consent to bioimpedance measures of my childs legs		**Yes**		**No**
I consent to circumferential measures of my childs legs		**Yes**		**No**
I consent to indurometry measures of my childs legs		**Yes**		**No**
I consent to ultrasound examination of my childs lymph nodes		**Yes**		**No**
I consent to a photograph of my childs legs		**Yes**		**No**

Name: *(printed)*	
Signature:	**Date:**

Appendix C2: Participant Information Sheet and Consent Form – Adult, Myanmar language

အသက်(၁၈)နှစ်အောက် သုတေသနတွင် ပါဝင်သူများ၏ မိဘ/အုပ်ထိန်းသူများထံမှ
သုတေသနတွင် ပါဝင်ရန်သဘောတူညီချက်တောင်းခံလွှာ

အဓိကသုတေသီ	ဂျန်(Jan Douglass)
ကျမ်းကြီးကြပ်သူ	တွဲဖက်ပါမောက္ခ Susan Gordon နှင့် Patricia Graves
တက္ကသိုလ်	ဂျိမ်စ်ကွတ်ခ်တက္ကသိုလ်၊James Cook University

ကျွန်ုပ်သည် လူငယ်များတွင် ဆင်ခြေဖော်ထောက်ရောဂါဆိုင်ရာသုတေသန လုပ်ငန်းများ ဆောင်ရွက်မည့် ဤသုတေသန၏ ရည်ရွယ်ချက်ကို သိရှိနားလည်ပြီးဖြစ်ပါသည်။ ကျွန်ုပ်နှင့် ကျွန်ုပ်၏ သား/သမီးအား ဤသုတေသနအကြောင်း ပြည့်စုံစွာရှင်းပြပြီးဖြစ်သည့်အတွက် ဤသုတေသနတွင် ကျွန်ုပ်၏သား/သမီးအား ပါဝင်ခွင့်ပြုရန် သဘောတူပြီးဖြစ်ပါသည်။ သုတေသနအကြောင်း သတင်းအချက်အလက်များစာရွက် ကိုလည်း ရရှိပြီးဖြစ်ပါသည်။ကျွန်ုပ်၏သား/သမီးအား သုတေသနတွင် မပါဝင်မီ လိုအပ်သော ကျန်းမာရေးအခြေအနေစစ်ဆေးမေးမြန်းမှုများ ပြုလုပ်ပြီးဖြစ်ပါသည်။ သုတေသနတွင်ပါဝင်သည့်အတွက် ကျွန်ုပ်၏သား/သမီး၏ လက်ထိပ်မှသွေးဖောက်ခြင်း၊ ခြေထောက်ကြွက်သားများတိုင်းတာသည့် ကိရိယာဖြင့် တိုင်းတာခြင်း၊ ခန္ဓာကိုယ်တွင်း ပြန်ရည်ကြောများတိုင်းတာသည့် ကိရိယာဖြင့်တိုင်းတာခြင်း၊ ခန္ဓာကိုယ်တွင်း ပြန်ရည်ကြောများ လှည့်ပတ်မှုကို တိုင်းတာသည့်ကိရိယာဖြင့်တိုင်းတာခြင်း၊ ယောကျ်ားလေးများ၏ ပေါင်ခြံတွင် သန်ကောင်များရှိ/မရှိ တယ်လီဗေးရှင်းဓါတ်မှန်စက်ဖြင့် ရှာဖွေခြင်းများ ပြုလုပ်မည်ကို သိရှိပါသည်။ တိုင်းတာမှုမှရရှိမည့် အဖြေရလဒ်များအား သုတေသီမှအသုံးပြုရန်သဘောတူပါသည်။ ကျွန်ုပ်သည် ကျွန်ုပ်၏ကလေးအား ဤသုတေသနတွင် မိမိဆန္ဒအလျောက်ပါဝင်ရန် သဘောတူခြင်းဖြစ်ပြီး၊ဤသုတေသနမှ တစုံတရာရှင်းလင်းပြောဆိုခြင်းမပြုဘဲအချိန်မရွေးနှုတ်ထွက် နိုင်ကြောင်းသိရှိပြီးဖြစ်ပါသည်။

ကျွန်ုပ်နှင့်ကျွန်ုပ်သား/သမီး၏ ကိုယ်ရေးအချက်အလက်များအား လျှို့ဝှက်ပေးထားမည်ဖြစ်ပြီး ကျွန်ုပ်၏ခွင့်ပြုချက်မရဘဲ အမည်၊ ကိုယ်ရေးအချက်အလက်များအား အသုံးပြုမည့်မဟုတ် ကြောင်းသိရှိပြီးဖြစ်ပါသည်။

	သဘောတူပါသည်။	သဘောမတူပါ
ကျွန်ုပ်သည် ကျွန်ုပ်သား/သမီး၏လက်ထိပ်မှ သွေးဖောက်ပြီး သွေးစစ် ကိရိယာကတ် နှင့် သွေးစစ်စက္ကူဖြင့် စမ်းသပ်ရန် သဘောတူ ပါသည်။		
ကျွန်ုပ်သည် ကျွန်ုပ်သား/သမီး၏ ခြေထောက်များအား ပေကြိုးဖြင့်တိုင်းတာရန် သဘောတူပါသည်။		
ကျွန်ုပ်သည် ကျွန်ုပ်သား/သမီး၏ ခြေထောက်များအား ကြွက်သားတိုင်းတာသည့်ကိရိယာဖြင့် တိုင်းတာရန်သဘောတူ ပါသည်။		
ကျွန်ုပ်သည် ကျွန်ုပ်သား/သမီး၏ ခန္ဓာကိုယ်ပြန်ရည်ကြော သွားလာမှုအား တယ်လီဗေးရှင်းဓါတ်မှန်ဖြင့် တိုင်းတာရန် သဘောတူပါသည်။		
ကျွန်ုပ်သည် ကျွန်ုပ်သား/သမီး၏ ခြေထောက်များအား ဓါတ်ပုံရိုက်ရန် သဘောတူပါသည်		

အမည်	
လက်မှတ်(သို့မဟုတ်) လက်ဗွေ (လက်ဝဲလက်မ)	ရက်စွဲ

Appendix C3: Participant Information Sheet and Consent Form – Child, Myanmar language

အသက်(၁၈)နှစ်အထက် သုတေသနတွင် ပါဝင်သူလူငယ်များထံမှ သုတေသနတွင်ပါဝင်ရန် သဘောတူညီချက်တောင်းခံလွှာ

အဓိကသုတေသီ	ဂျန်(Jan Douglass)
ကျမ်းကြီးကြပ်သူ	တွဲဖက်ပါမောက္ခ Susan Gordon နှင့် တွဲဖက်ပါမောက္ခ Patricia Graves
တက္ကသိုလ်	ဩစတြေးလျနိုင်ငံ၊ James Cook Univusity

ကျွန်ုပ်သည် လူငယ်များတွင် ဆင်ခြေထောက်ရောဂါဆိုင်ရာသုတေသန လုပ်ငန်းများ ဆောင်ရွက်မည့် ဤသုတေသန၏ ရည်ရွယ်ချက်ကို သိရှိနားလည်ပြီးဖြစ်ပါသည်။ ကျွန်ုပ်အား ဤသုတေသနအကြောင်း ပြည့်စုံစွာရှင်းပြပြီးဖြစ်သည့်အတွက် ဤသုတေသနတွင် ပါဝင်ရန် သဘောတူပြီးဖြစ်ပါသည်။သုတေသနအကြောင်း သတင်းအချက်အလက်များ စာရွက်ကိုလည်း ရရှိပြီးဖြစ်ပါသည်။ ကျွန်ုပ်အား သုတေသနတွင်မပါဝင်မီလိုအပ်သော ကျန်းမာရေးအခြေအနေ စစ်ဆေးမေးမြန်းမှုများ ပြုလုပ်ပြီးဖြစ်ပါသည်။သုတေသနတွင် ပါဝင်သည့်အတွက် ကျွန်ုပ်အား လက်ထိပ်မှသွေးဖောက်ခြင်း၊ ခြေထောက်ကြွက်သားများတိုင်းတာသည့် ကိရိယာဖြင့် တိုင်းတာခြင်း၊ ယောကျ်ားကလေးများ၏ ပေါင်ခြံတွင် သန်ကောင်များရှိ/မရှိ တယ်လီဇေးရှင်းဓါတ်မှန်စက်ဖြင့် ရှာဖွေခြင်းများပြုလုပ်မည်ကိုသိရှိပါသည်။ တိုင်းတာမှုမှရရှိမည့် အဖြေရလဒ် များအား သုတေသီမှအသုံးပြုရန်သဘောတူပါသည်။

ကျွန်ုပ်သည် သုတေသနတွင် မိမိဆန္ဒအလျောက်ပါဝင်ခြင်းဖြစ်ပြီး၊ ဤသုတေသနမှ တစုံတရာရှင်းလင်း ပြောဆိုခြင်းမပြုဘဲအချိန်မရွေးနှုတ်ထွက် နိုင်ကြောင်းသိရှိပြီးဖြစ်ပါသည်။

ကျွန်ုပ်၏ ကိုယ်ရေးအချက်အလက်များနှင့် သုတေသနပြုလုပ်ရာမှတွေ့ရှိသည့် အချက်အလက်များအား လျှို့ဝှက်ပေးထားမည်ဖြစ်ပြီး ကျွန်ုပ်၏ခွင့်ပြုချက်မရဘဲ အမည်၊ ကိုယ်ရေးအချက်အလက်များ အား အသုံးပြုမည်မဟုတ်ကြောင်းသိရှိပြီးဖြစ်ပါသည်။

	သဘောတူပါသည်။	သဘောမတူပါ
ကျွန်ုပ်သည် ကျွန်ုပ်သား/သမီး၏လက်ထိပ်မှ သွေးဖောက်ပြီး သွေးစစ်ကိရိယာကတ် နှင့် သွေးစစ်စက္ကူဖြင့် စမ်းသပ်ရန် သဘောတူပါသည်။		
ကျွန်ုပ်သည် ကျွန်ုပ်၏ သွေးနမူနာ(၁၀)စီစီခန့် ထုတ်ယူရန် သဘောတူပါသည်။		
ကျွန်ုပ်သည် ကျွန်ုပ်၏ ခြေထောက်များအား ပေကြိုးဖြင့် တိုင်းတာရန် သဘောတူပါ သည်။		
ကျွန်ုပ်သည် ကျွန်ုပ်၏ ခြေထောက်များအား ကြွက်သား တိုင်းတာသည့် ကိရိယာဖြင့် တိုင်းတာရန် သဘောတူပါသည်။		
ကျွန်ုပ်သည် ကျွန်ုပ်၏ ခန္ဓာကိုယ်ပြန်ရည်ကြော သွားလာမှုအား တယ်လီပေးရှင်း ဓါတ်မှန်ဖြင့် တိုင်းတာရန် သဘောတူပါသည်။		
ကျွန်ုပ်သည် ကျွန်ုပ်၏ ခြေထောက်များအား ဓါတ်ပုံရိုက်ရန် သဘောတူပါသည်။		

အမည်	
လက်မှတ်(သို့မဟုတ်) လက်ဗွေ (လက်ဝဲလက်မ)	ရက်စွဲ

Appendix C4: Participant Information Sheet and Consent Form – Adult, Australia

Participant Information Sheet

PROJECT TITLE: **A study of the user reliability of four devices to measure tissue compressibility, fluid volumes and skin thickness in the legs of young people.**

You are invited to take part in a research project to determine if four devices for measuring legs are reliable. The first device is called an Indurometer and is a hand held unit that applies a 200gm weight to a small area of skin – about the same amount of pressure as having your pulse taken. The second unit is called a Skin Fibro Meter and has a similar action, applying a measured 50gm pressure to the skin. The third device is bio impedance spectroscopy (BIS) which uses a low voltage current to detect fluid levels in the soft tissue just under the skin. The current is too low to be felt and it works in a similar way to the digital bathroom scales that give a measure of fat and muscle mass. The fourth device is a high frequency ultrasound which can measure the skin thickness similar to other ultrasound devices used in clinical imaging. All four devices are used to measure changes in skin resistance or thickness and increased fluid loads in adults who have problems with their lymphatic system. We want to use them to measure similar changes in young people who may have these problems but first we need to see if they can accurately measure skin and underlying fluid in young people with normal legs. None of the devices cause any pain or discomfort other than the pressure felt when the 200 gram weight is applied to the skin (Indurometer and Skin Fibro Meter) or during application and removal of the adhesive electrodes (BIS) or the ultrasound probe.

If you agree to be involved in the study you will be invited to attend JCU to be interviewed and have the measurements taken. The interview will include questions about your general health including; age, gender, any family history of fluid retention and the onset of puberty. If you are female you will be asked about the current stage of your menstrual cycle since this can influence the build-up of fluids.

Height and weight measurements will be taken using scales similar to those you would find in a hospital or health clinic. You will then be asked to lie face up on a padded table with both legs uncovered. The midpoint of each upper leg between the top of the knee and the crease in the groin will be located with a tape measure and marked with a non-permanent pencil – similar to a lip liner pencil. The circumference of each thigh will be measured with a tape measure. The Indurometer is a small hand held device with a short plunger extending from a flat Perspex plate and will be used to measure the amount the tissue can be compressed at the midpoint marker. The plate is laid flat on the skin over the marked point and pressed gently into the skin. The device will emit a short beep when 200 grams of pressure has been applied and the measure has been recorded. The Skin Fibro Meter is a small hand held device which is placed over the marked area and delivers a measured 50gm pressure. The high frequency ultrasound will also be used over the marked area. A small amount of a water based gel will be used and the ultrasound probe will be gently applied to the skin. Deep pressure is not applied during the ultrasound procedure.

You will then be asked to turn face down and the process will be repeated finding the midpoint of the thigh between the crease at the top of the leg and the crease behind the knee, and the midpoint of the calf between the crease behind the knee and the crease at the top of the ankle. A tape measurement will be used to measure the circumference of the calf and the Indurometer, Skin Fibro Meter and ultrasound will be used at the midpoint of each thigh and each calf.

You will be asked to turn face up again and the self-adhesive electrodes for the BIS will be attached. These will be placed at the top of the leg near the crease of the groin, the inside of the upper shin just below each knee, the top of each foot and the back of each hand. Electrical leads will then be attached to the electrode tabs and a low level current will be passed through the body. The BIS device is a battery operated device and the low level current cannot be felt by the person. After the measurement has been taken the electrodes will be removed and you will be given a wet wipe to remove any adhesive residue or non-permanent marker pen remaining on the skin

If you agree to be involved in the study you will be asked to drink only plain water and not to undertake any vigorous exercise for 2 hours prior to the measuring appointment. Since the measurements will be taken on the thighs and calves it would be ideal to wear clothing that allows access to these body areas such as sports shorts or bathers. Your modesty will be preserved at all times. You will be asked to remove any metal jewellery or studs and so may prefer to remove these before attending the appointment.

Each of the measurements with the tape measure, the Indurometer, the Skin Fibro Meter, the high frequency ultrasound and the BIS will be repeated three times so that an average can be calculated. Some measures may need to be repeated more often if the results are not clear. It is anticipated that the interview and measuring procedures will take about one hour of your time. The interview and measurement procedures will be conducted at the School of Public Health, Tropical Medicine and Rehabilitation Sciences at James Cook University.

Taking part in this study is completely voluntary and you can stop taking part in the study at any time without explanation or prejudice. If you do not give verbal agreement at the time for any measurement procedure to be performed that procedure will not be performed.

If you know of others that might be interested in this study, please pass this information sheet to them so they can contact me to volunteer for the study.

Your responses and contact details will be strictly confidential. The data from the study will be used in research publications, reports and theses and at conference presentations. You will not be identified in any way in these publications or presentations.

The study is being conducted by Jan Douglass and will contribute to her Doctoral research project in Public Health at James Cook University (JCU).

If you have any questions about the study, please contact Jan Douglass on 0419 848 589, 4781 6680 or jan.douglass@my.jcu.edu.au

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Human Ethics, Research Office
James Cook University, Townsville, Qld, 4811
Phone: (07) 4781 5011 (ethics@jcu.edu.au)

INFORMED CONSENT FORM – Young adult

PRINCIPAL INVESTIGATOR: Jan Douglass
PROJECT TITLE: A study of the user reliability of four devices to measure tissue compressibility,fluid volumes and skin thickness in the legs of young people.
SCHOOL: Public Health, Tropical Medicine and Rehabilitation Sciences

I understand the aim of this research study is to test the reliability of four devices that measure tissue compression (Indurometer and Skin Fibro Meter) fluid volumes in the legs (Bioimpedance Spectroscopy) and skin thickness (high frequency ultrasound).understand the aim of this research study is to test the reliability of a device that measures tissue compression (Indurometry) and a device that measures fluid volumes in the legs (Bioimpedance Spectroscopy).

I consent to my participation in this project, the details of which have been explained to me, and I have been provided with a written information sheet to keep.

I understand that my participation will involve an interview and some measuring procedures and I agree that the researcher may use the results as described in the information sheet.

I acknowledge that:

- taking part in this study is voluntary and I am aware that I can stop taking part in it at any time without explanation or prejudice and to withdraw any unprocessed data I have provided;

- that any information I give about myself will be kept strictly confidential and that no names will be used to identify me with this study without my approval;

	(Please tick to indicate consent)			
I consent to be interviewed		**Yes**		**No**
I consent to my height, weight, calf and thigh circumferences being measured		**Yes**		**No**
I consent to my thighs and calves to be measured with an Indurometer (a 200 gm weight will be applied to the skin of the thighs front and back and both calves)		**Yes**		**No**
I consent to my thighs and calves to be measured with a Skin Fibro Meter (a 50 gm weight will be applied to the skin of the thighs front and back and both calves)		**Yes**		**No**
I consent to my thighs and calves to be measured with an high frequency ultrasound (an ultrasound probe will be applied to the skin of the thighs front and back and both calves)		**Yes**		**No**
I consent to my thighs and calves to be measured with bio impedance spectroscopy (BIS) (self-adhesive electrodes will be applied to the top of the foot, knee and thigh of each leg and the back of each hand and an imperceptible low voltage electrical current will be passed through the body)		**Yes**		**No**

Name: *(printed)*	
Signature:	**Date:**

Appendix C5: Participant Information Sheet and Consent Form – Child, Australia

JAMES COOK UNIVERSITY AUSTRALIA

INFORMATION SHEET – Parent of a Child or Adolescent

PROJECT TITLE: **A study of the user reliability of two devices to measure tissue compressibility and fluid volumes in the legs of young people.**

Your child is invited to take part in a research project to determine if four devices for measuring legs are reliable. The first device is called an Indurometer and is a hand held unit that applies a 200gm weight to a small area of skin – about the same amount of pressure as having your pulse taken. The second unit is called a Skin Fibro Meter and has a similar action, applying a measured 50gm pressure to the skin. The third device is bio impedance spectroscopy (BIS) which uses a low voltage current to detect fluid levels in the soft tissue just under the skin. The current is too low to be felt and it works in a similar way to the digital bathroom scales that give a measure of fat and muscle mass. The fourth device is a high frequency ultrasound which can measure the skin thickness. All four devices are used to measure changes in skin resistance and fluid loads in adults who have problems with their lymphatic system. We want to use them to measure similar changes in young people who may have these problems but first we need to see if they can accurately measure skin and underlying fluid in young people with normal legs. None of the devices cause any pain or discomfort other than the pressure felt when the 200 gram weight is applied to the skin (Indurometer and Skin Fibro Meter) or during application and removal of the adhesive electrodes (BIS) or the ultrasound probe.

If you agree for your child to be involved in the study you and your child will be invited to attend JCU to be interviewed and have the measurements taken. The interview will include questions about your child's general health including; age, gender, any family history of fluid retention and the onset of puberty. If your child is female you will be asked about the current stage of her menstrual cycle since this can influence the build-up of fluids. You will be asked to remain with your child throughout the interview and measurement procedures.

Height and weight measurements will be taken using scales similar to those you would find in a hospital or health clinic. Your child will then be asked to lie face up on a padded table with both legs uncovered. The midpoint of each upper leg between the top of the knee and the crease in the groin will be located with a tape measure and marked with a non-permanent pencil – similar to a lip liner pencil. The circumference of each thigh will be measured with a tape measure. The Indurometer is a small hand held device with a short plunger extending from a flat Perspex plate and will be used to measure the amount the tissue can be compressed at the midpoint marker. The plate is laid flat on the skin over the marked point and pressed gently into the skin. The device will emit a short beep when 200 grams of pressure has been applied and the measure has been recorded. The Skin Fibro Meter is a small hand held device which is placed over the marked area and delivers a measured 50gm pressure. The high frequency ultrasound will also be used over the marked area. A small amount of a water based gel will be used and the ultrasound probe will be gently applied to the skin. Deep pressure is not applied during the ultrasound procedure.

Your child will then be asked to turn face down and the process will be repeated finding the midpoint of the thigh between the crease at the top of the leg and the crease behind the knee, and the midpoint of the calf between the crease behind the knee and the crease at the top of the ankle. A tape measurement will be used to measure the circumference of the calf and the Indurometer, Skin Fibro Meter and ultrasound will be used at the midpoint of each thigh and each calf.

Your child will be asked to turn face up again and the self-adhesive electrodes for the BIS will be attached. These will be placed at the top of the leg near the crease of the groin, the inside of the upper shin just below each knee, the top of each foot and the back of each hand. Electrical leads will then be attached to the electrode tabs and a low level current will be passed through the body. The BIS device is a battery operated device and the low level current cannot be felt by the person. After the measurement has been taken the electrodes will be removed and you will be given a wet wipe to remove any adhesive residue or non-permanent marker pen remaining on the skin

If you agree for your child to be involved in the study you will be asked to ensure that you child drinks only plain water and does not undertake any vigorous exercise for 2 hours prior to the measuring appointment. Since the measurements will be taken on the thighs and calves it would be ideal for your child to wear clothing that allows access to these body areas such as sports shorts or bathers. Your child's modesty will be preserved at all times and you will be expected to stay with your child throughout all procedures. Your child will be asked to remove any metal jewellery or studs and so may prefer to remove these before attending the appointment.

Each of the measurements with the tape measure, the Indurometer, the Skin Fibro Meter, the high frequency ultrasound and the BIS will be repeated three times so that an average can be calculated. Some measures may need to be repeated more often if the results are not clear. It is anticipated that the interview and measuring procedures will take about an hour of your time. The interview and measurement procedures will be conducted at the School of Public

Health, Tropical Medicine and Rehabilitation Sciences at James Cook University.

Taking part in this study is completely voluntary and your child can stop taking part in the study at any time without explanation or prejudice. If you or your child do not give verbal agreement at the time for any measurement procedure to be performed that procedure will not be performed.

If you know of others that might be interested in this study, please pass this information sheet to them so they can contact me to volunteer for the study.

Your responses and contact details will be strictly confidential. The data from the study will be used in research publications, reports and theses and at conference presentations. You or your child will not be identified in any way in these publications or presentations.

The study is being conducted by Jan Douglass and will contribute to her Doctoral research project in Public Health at James Cook University (JCU).

If you have any questions about the study, please contact Jan Douglass on 0419 848 589, 4781 6680 or jan.douglass@my.jcu.edu.au

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If you have any concerns regarding the ethical conduct of the study, please contact:
Human Ethics, Research Office
James Cook University, Townsville, Qld, 4811
Phone: (07) 4781 5011 (ethics@jcu.edu.au)

INFORMED CONSENT FORM – Parent of a Child or Adolescent

PRINCIPAL INVESTIGATOR: Jan Douglass
PROJECT TITLE: A study of the reliability of two devices to measure tissue compressibility and fluid volumes in the legs of young people
SCHOOL: Public Health, Tropical Medicine and Rehabilitation Sciences

I understand the aim of this research study is to test the reliability of a device that measures tissue compression (Indurometer and Skin Fibro Meter, a device that measures fluid volumes in the legs (Bioimpedance Spectroscopy) and high frequency ultrasound to measure the skin thickness.

I consent to my child's participation in this project, the details of which have been explained to me, and I have been provided with a written information sheet to keep.

I understand that my child's participation will involve an interview and some measuring procedures and I agree that the researcher may use the results as described in the information sheet.

I acknowledge that:

- taking part in this study is voluntary and I am aware that I can stop my child from taking part in it, at any time without explanation or prejudice and to withdraw any unprocessed data provided;

- that any information I give about my child will be kept strictly confidential and that no names will be used to identify my child with this study without my approval;

	(Please tick to indicate consent)			
I consent to be interviewed		**Yes**		**No**
I consent to my child's height, weight, calf and thigh circumferences being measured		**Yes**		**No**
I consent to my child's thighs and calves to be measured with an Indurometer (a 200 gm weight will be applied to the skin of the thighs front and back and both calves)		**Yes**		**No**
I consent to my child's thighs and calves to be measured with a Skin Fibro Meter (a 50 gm weight will be applied to the skin of the thighs front and back and both calves)		**Yes**		**No**
I consent to my child's thighs and calves to be measured with an high frequency ultrasound (an ulatrasound probe will be applied to the skin of the thighs front and back and both calves)		**Yes**		**No**
I consent to my child's thighs and calves to be measured with bio impedance spectroscopy (BIS) (self-adhesive electrodes will be applied to the top of the foot, knee and thigh of each leg and the back of each hand and an imperceptible low voltage electrical current will be passed through the body)		**Yes**		**No**

Name: *(printed)*	
Signature:	**Date:**

Appendix D: Recruitment and screening, Australia

Appendix D1: Recruitment poster

Appendix D2: Phone interview form

Appendix D1: Recruitment poster

Appendix D2: Phone interview form

Phone Screening Form. JD reliability study

*Name*_____*Phone number*_____

*Email*_____*Date* _____

☐ *Young adult (18 – 21) DOB*_____

☐ *Parent of a minor (8 - 17) Name of child* _____ *DOB* _____

✓ box for yes, X for no

Any X = cannot be included

☐ Age 8 - 21

☐ Able to understand the study protocol or comply with the study procedures

☐ Willing to comply with the restriction to consume only water for 2 hours prior to appointment.

☐ Willing to comply with the restriction not to undertake excessive exercise for 2 hours prior to appointment

☐ Able to attend JCU for a single measurement sessions (not more than 1 hour total)

☐ Able to give informed consent for self/child (cross one out)

✓ box for yes, X for no
Any ✓ = cannot be included

☐ Have a known heart condition or any implanted electrical devices such as a pacemaker

☐ Have any surgically implanted metal device such as prosthetic joints not including small implants such as sternal wires or surgical staples

☐ Suffer from a renal disorder

☐ Currently have a reported fever of >38C

☐ Pregnant or breast feeding

☐ Have open areas of skin on the lower limb or back of the hands

☐ Have an acute injury to the lower limbs

☐ Have any condition which precludes lying in the supine and prone positions for 15 minutes

☐ Inability to understand patient information sheet or give informed consent

☐ *Not eligible for participation. Notified Yes / No date* _____

☐ *Eligible for participant. Trial participant number*_____

Menstruating females only, date of onset of last menstrual period _____

Appendix E: Interview sheets and bio data forms

Appendix E1: Participant Medical and Personal History questionnaire

Appendix E2: Biodata form

Appendix E1: Participant Medical and Personal History questionnaire

Participant Medical and Personal History

Date of interview

Staff member

Name of child

Address or contact details

Name of Parent/s

Personal Details

DOB or age today

Medical History

Has your child had any surgery to their legs, tummy or groin area?

No

Yes

List (check for metal implants and pacemaker)

Does your child have any medical conditions?

No

Yes

List

Does your child take any medication?

No

Yes

List

Has your child been unwell recently?

No

Yes

Describe symptoms/when/length of illness

Based on these responses this child is / is not eligible to participate in the study

Other questions to be included which may alter data

Which leg would you kick a ball with? Right Left

Does anyone in your family have swelling in their leg or legs?

No

Yes

List

Relationship to you _____

Affected leg L / R / both. Grade or description (if known) _____

Did they take the MDA drugs in 2014?

Yes

No

Don't know

Did they take the deworming medication in 2015

Yes

No

Date medication was commenced .../.../2015

Number of days medication was consumed _____days

Number of pills left over _____

Lymphoedema History

Initial visual assessment. Does either leg look swollen?

No

Yes

Grade of swelling

Position of swelling global discrete area (describe)

Skin condition hyperpigmentation dry scaley other

Are any skin lesions present?

No

Yes

Type of lesion	ulcer	open wound	interdigital lesions	cracks/fissures	
Location	left	right	malleoli	soles	ankle
Description	clean	infected			

If the leg/s look swollen

Have you ever had an infection in the leg

NONE

ONE

LESS THAN 5

5-10

GREATER THAN 10

DON'T KNOW

Details of infection control:

_____

_____

Progression : Worse in evenings Y / N _____

Changes with weather, worse when hot Y / N _____

Other aggravating factors _____

Other alleviating factors _____

Past History :

Prior tissue trauma to the affected limb Y / N

Details_____

Prior leg breakage Y / N

Details _____

Bad sunburn legs at some time Y / N

Details_____

Insect (spider/ant/bee) bite to region Y / N

Details_____

Any other injury to limb/area Y / N

Details_____

Appendix E2: Biodata form

Date 11/6/15	RA	LF

Participant Personal and Family History

Name				
Village			Phone	
DOB		Age		
Gender	M / (F)	Occupation	Student Construction Weaving Street Vendor	Other ✓
Past Surgery	Yes / (No)	Metal Implant or Pacemaker	Yes / (No)	Details
Medical Problem	Yes / (No)	Regular Medicines	Yes / (No)	Details
Recent Illness	Yes / (No)			
Last drink	<30 <60 <120 (>120)		Female only	
Last food	<30 <60 <120 (>120)		Last menstrual period	N/A < 7 days > 7 days > 14 days ✓
Last bladder void	<30 <60 <120 (>120)			
Kicking leg	(Right) / Left	MDA 2014	Yes / (No) / Don't Know	
Drug treatment 2015	Yes / (No)	Number of days	1 2 3 4 5 6 7 8 9 10 11 12	
Does anyone in your family have a big leg?	Yes / (No)	Who?	Description of leg	Duration < 1yr <5 year >10 years
Affected leg	L R Both DK	Treatment		

214

Date 11 / 6 / 15	RA	LF

Physical Measures

Height	155 Cm	Weight	36.2 Kg

	Calf R	Calf L	Post Thigh R	Post Thigh L	Ant Thigh R	Ant Thigh L
Length	43	43	27	27	30	30
Midpoint	21.5	21.5	13.5	13.5	15	15

Calf	SFM R	0.12	0.10	
	SFM L	0.09	0.10	
	IND R	2.70	2.96	
	IND L	2.53	2.58	
	TON R	4.21	4.49	
	TON L	3.96	4.19	
	Circ R	23.2		
	Circ L	23.2		
Thigh Back	SFM R	0.08	0.08	
	SFM L	0.05	0.04	
	IND R	4.74	5.20	
	IND L	4.27	4.02	
	TON R	7.16	7.04	
	TON L	7.38	7.44	
Thigh Front	SFM R	0.05	0.05	
	SFM L	0.06	0.06	
	IND R	5.69	5.84	
	IND L	4.44	4.78	
	TON R	8.65	8.69	
	TON L	6.36	6.94	
	Circ R	36.6		
	Circ L	35.7		

Appendix F: Publications

Appendix F1: Systematic Review

Appendix F2: Reliability study

Appendix F3: Moderating factors in device measures

Appendix F4: Cross-sectional analysis – Myanmar

Appendix F5: List of oral and poster presentations

Appendix F1: Systematic Review

Douglass, J., Graves, P., Gordon, S., *Self-Care for Management of Secondary Lymphedema: A Systematic Review.* PLoS Neglected Trop Dis, 2016. **10**(6): p. e0004740.

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RESEARCH ARTICLE

Self-Care for Management of Secondary Lymphedema: A Systematic Review

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OPEN ACCESS

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Abstract

Background

Lymphedema is a debilitating and disfiguring sequela of an overwhelmed lymphatic system. The most common causes of secondary lymphedema are lymphatic filariasis (LF), a vector-borne, parasitic disease endemic in 73 tropical countries, and treatment for cancer in developed countries. Lymphedema is incurable and requires life-long care so identification of effective lymphedema management is imperative to improve quality of life, reduce the burden on family resources and benefit the local community. This review was conducted to evaluate the evidence for effective lymphedema self-care strategies that might be applicable to management of all types of secondary lymphedema.

Methodology/Principal Findings

Searches were conducted in Medline, CINAHL and Scopus databases in March 2015. Included studies reported before and after measures of lymphedema status or frequency of acute infections. The methodological quality was assessed using the appropriate Critical Appraisal Skills Program checklist. Descriptive synthesis and meta-analysis were used to evaluate effectiveness of the outcomes reported. Twenty-eight papers were included; two RCTs were found to have strong methodology, and overall 57% of studies were rated as methodologically weak. Evidence from filariasis-related lymphedema (FR-LE) studies indicated that hygiene-centred self-care reduced the frequency and duration of acute episodes by 54%, and in cancer-related lymphedema (CR-LE) home-based exercise including deep breathing delivered significant volume reductions over standard self-care alone. Intensity of training in self-care practices and frequency of monitoring improved outcomes. Cultural and economic factors and access to health care services influenced the type of intervention delivered and how outcomes were measured.

Conclusions/Significance

There is evidence to support the adoption of remedial exercises in the management of FR-LE and for a greater emphasis on self-treatment practices for people with CR-LE. Empowerment of people with lymphedema to care for themselves with access to supportive professional assistance has the capacity to optimise self-management practices and improve outcomes from limited health resources.

Author Summary

Secondary lymphedema is a major cause of disability worldwide. The most common causes are treatment for cancer or infection with lymphatic filariasis. In both cases, the lymphatic system is damaged and unable to perform its normal function of removing extracellular fluid and wastes. Protein rich fluid accumulates in the affected area, and if left untreated may progress to 'elephantiasis', which is characterised by a grossly enlarged limb and thickening of the skin. Lymphedema is incurable, requires lifelong care, and psychosocial support, but early intervention and good self-management practices can halt progression, preserve quality of life and maintain the ability to participate in normal work and social activities. Current approaches to treatment vary depending on the setting. In developed countries, cancer related lymphoedema is therapist-based and aims to intervene early and prevent disease progression. Lymphatic filariasis related lymphedema is associated with poverty, affecting people living in developing countries where minimal intervention is recommended or available for early stages. By identifying useful practices that can be transferred across cultural and economic borders, people living with lymphedema can be empowered to care for themselves and improve their long term outcomes.

Introduction

Lymphedema is a high protein edema which forms when the lymphatic system is chronically overwhelmed. Earlier fluid rich stages progress gradually toward enlargement and fibrosis of the subcutaneous compartment and hyperkeratosis of the skin (elephantiasis) [1]. This can occur as a result of congenital factors (primary lymphedema) but is more commonly caused by an alteration in normal lymphatic function leading to secondary lymphedema. The majority of secondary lymphedema occurs through infection with a vector-borne, parasitic disease known as lymphatic filariasis (LF) which is endemic in 73 tropical countries where is it closely associated with poverty [2]. In developed countries lymphedema is more commonly a consequence of some cancer treatments which involve lymph node removal or irradiation. Global estimates of filariasis-related lymphedema (FR-LE) are 16.7 million cases [3] and cancer-related lymphedema (CR-LE) is estimated to affect between 15% and 80% of all cancer survivors [4]. Annual mass drug administration (MDA) of anti-filarial chemotherapy can prevent future transmission of LF [5] and improvements in surgical management of cancer should reduce the incidence of new CR-LE cases [6] but in both aetiologies onset of chronic symptoms may be delayed for months, years or even decades after exposure to the risk [4, 7]. People with any impairment to lymphatic function bear a lifelong risk of developing secondary lymphedema [7].

Lymphatic vessels remove circulating fluid and large molecules from the extracellular spaces of almost all body tissues and transport them to the lymph nodes. This is essential for

continuous clearance of pathogenic elements crossing the skin barrier and entering the subcutaneous compartment and in other tissues it is vital in maintaining correct extracellular fluid balance. Cleaned and filtered lymph is returned to systemic circulation via the vascular system. In lymphedema, when normal lymph transport is impeded, protein rich fluid accumulates, mostly in the subcutaneous compartment. Risk of infection is increased; namely acute dermato-lymphangio-adenitis (ADLA) in FR-LE, and cellulitis or erysipelas in CR-LE. Infection then exacerbates disease progression and as lymphedema advances, symptoms become increasingly disabling and disfiguring [8]. In areas endemic for LF, lymphedema causes social stigma, superstition and loss of opportunity to marry [9]. People with CR-LE report depression, poor quality of life and an inability to engage in paid employment [10].

Although essentially the same chronic disease, treatments for FR-LE and CR-LE follow different guidelines. The World Health Organization recommends community based home care (CBHC)[5, 11–13] to improve hygiene and reduce ADLA episodes (the main cause of lost working days) in FR-LE. The program promotes frequent washing and drying of affected areas with particular attention to entry lesions (potential sites of fungal and bacterial infection), passive elevation and range of motion (ROM) exercises, and the use of oral antibiotic or anti-inflammatory medications during acute events. Self-massage and compression bandaging are recommended in advanced stages but usually not implemented in resource-poor settings [14]. In contrast the gold standard for CR-LE is a two phase program with an initial, intensive period of therapist based treatment applying specialized lymphatic massage and multilayer compression bandaging to reduce limb size; followed by an ongoing maintenance phase of self (or partner) lymphatic massage with regular use of compression garments [15]. Meticulous skin care and remedial exercises are a component of both phases.

Established lymphedema is considered to be irreversible, necessitating lifelong care, and family and psychosocial support [1]. Effective management of either FR-LE or CR-LE can improve quality of life for the individual, reduce the burden on family resources and benefit the local community. In developing countries there may be few or no health care services available for people with FR-LE and resources allocated to CR-LE treatment in many developed countries are also insufficient [16, 17]. Poor access to lymphedema health services in both settings and limited evidence of treatment efficacy has resulted in misinformation and unproven management practices. Approaches to management will ultimately be shaped by access to resources and cultural, financial and political influences [17], but effective core strategies that can be applied across cultural and economic borders need to be identified. This review was undertaken to evaluate the outcome of current self-care interventions for FR-LE and CR-LE. Differences and similarities were assessed with respect to self-care components included in the intervention, outcome measures used and the extent of any support services and monitoring. The results have illuminated beneficial practices that may inform health systems in any setting to increase the effectiveness of self-care strategies for people with secondary lymphedema.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [18] guided the methodology of this review (protocol registration number CRD42013004850). The research question and search terms were determined using the PICO model (population, intervention, comparison, outcomes) [19]. **Participants** were defined as people with established FR-LE or CR-LE. **Interventions** were any type of self-care procedure that could be performed by an individual or within the family group and these included different self-care protocols or specific components of self-care such as self-massage or resistance exercise. **Comparisons** were either between groups in studies with more than one study group

performing self-care, or before- and -after cohort studies (controlled or uncontrolled). **Outcomes** were at least one of either a change in objective measure of lymphedema status (such as lymphedema stage, limb volume or limb circumference) or change in frequency or duration of acute episodes (ADLA or cellulitis). Additional optional outcomes were functional or perceived disability, and self-reported symptoms.

Search strategy

Searches were conducted in Medline (OVID), CINAHL and Scopus databases in October 2013 and updated during March 2015. Medical Subject Headings (MeSH) were used in the Medline search and keywords for the Scopus and CINAHL searches were derived from the Medline MeSH terms to ensure consistency of search terms (Table 1). The search strategy was limited to publications in English and grey literature was not searched. Reference lists of studies and reviews, World Health Organization (WHO) summary reports and editorials were searched to find other original peer reviewed studies. After removal of duplicates the title and abstract of returned studies were screened by two authors (JD, SG). Full inclusion and exclusion criteria used are detailed in S1 Tables.

Types of studies. The Australian National Health and Medical Research Council (NHMRC) hierarchy of evidence [20] advocates randomised controlled trial (RCT) as the most appropriate study design to answer clinical questions such as the effectiveness of self-care for lymphedema. However, ethical issues may prevent comparison of basic self-care to no self-care and no such trials were found. Therefore, this review included randomized or quasi-randomized comparisons of different types of self-care, as well as before and after cohort studies (controlled or uncontrolled). Studies, including RCTs, where the primary outcome was assessment of a drug or therapist based intervention were accepted if before and after measures for any self-care group(s) was included. When such a study had only one group which met the inclusion criteria for the review, the results of this group were considered during data extraction as for an uncontrolled before and after cohort study.

Studies were excluded if they employed electrical devices, compression pumps or any equipment such as swimming pools which would not be readily available in an average household. Studies were also excluded if the intervention was not solely self-care i.e. required a surgical procedure, was applied by a therapist, or if exercises were performed in instructor led classes. Studies where the primary outcome was quality of life (QOL), socioeconomic factors or evaluation of program implementation were included only if they also reported objective measures of lymphedema status or frequency of infection.

Table 1. Keywords used to search databases.

Search	Keywords
Search 1	((lymphoedema or lymphedema or elephantiasis) and (filariasis))
Search 2	((lymphoedema or lymphedema or elephantiasis) and (cancer or oncology))
Search 3	1 OR 2
Search 4	(self-care or "self care" or basic-care or "basic care" or "community based home care" or "community-based home-care" or "self management" or self-management or self-treatment or "self treatment" or self-massage or "self massage" or "partner massage" or home-care or "home care" or limb-care or "limb care" or foot-care or "foot care" or hygiene or breathing or exercise)
Search 5	3 AND 4

doi:10.1371/journal.pntd.0004740.t001

Assessment of study quality

Two authors (JD, SG) independently appraised the included studies for methodological quality using the Critical Appraisals Skill Program (CASP) RCT appraisal checklist or cohort appraisal checklist appropriate to the study design [21]. A rating of strong, moderate or weak was awarded to each study according to the number of 'yes', 'can't tell' and 'no' responses. Studies without 'no' or with two or less 'can't tell' answers were considered methodologically strong. Studies with one 'no' or three 'can't tell' answers were rated moderate and studies with two or more 'no' answers rated weak [22]. When the independent appraisal process was completed, studies which did not achieve the same rating by both reviewers were discussed until agreement was reached. A third reviewer (PG) was available where discrepancies could not be resolved but was not required.

Data extraction and synthesis

One author (JD) independently extracted study data including number of participants, intervention, outcomes, outcome measures, and results. Special characteristics of gender, age, country/setting, lymphedema cause, affected site and stage (or grade) of lymphedema were noted. Data were extracted for change in lymphedema status and effect on acute episodes. Where results were reported consistently with appropriate estimate of precision, a meta-analysis was performed using Review Manager version 5.3 (The Nordic Cochrane Centre, 2014). Data on effect size and outcomes of clinical importance were extracted for narrative synthesis and to highlight differences between the FR-LE and CR-LE studies.

Results

Combined searches including reference lists, hand searches and expert referrals yielded 1008 unique articles of which 940 were excluded by title or abstract. Sixty-eight full text articles were screened with 28 meeting the criteria for inclusion. Fig 1 shows the PRISMA flow diagram of study selection and details exclusions by criteria.

Study design

Ten RCT's including one quasi RCT and one randomized cross over trial were included. Of these, three FR-LE trials [23–26] and two CR-LE trials [27, 28] compared two or more groups performing different self-care protocols. The remaining five RCTs included only one group using self-care alone and these results were considered as uncontrolled, prospective cohorts [29–33]. Most cohort studies were prospective: ten on FR-LE, including two reports on the same cohort [34, 35], and four on CR-LE. There was one retrospective follow up on a previous FR-LE RCT and two retrospective reports on CR-LE.

Quality rating and risk of bias. Two RCT's were rated as strong but only one of these compared two groups that could be included for review [24]. Of the five RCTs rated as moderate one had two groups performing self-care [27] and four included only one group which met the inclusion criteria. The main risks of bias among the RCTs were inadequate blinding, disparity of participants at baseline and unequal treatment of groups. No cohort studies were rated as methodologically strong and five were rated as moderate quality [35–39]. Risks of bias included: recruitment by convenience sampling, insufficient consideration of confounding factors, low measurement sensitivity and large loss to follow up. Overall 16 studies (57%) were rated as weak methodological quality. The methodological quality rating of each study is provided in S1 Tables.

Population

There were eighteen reports on seventeen studies about FR-LE and all were conducted in tropical countries endemic for *Brugia malayi* or *Wuchereria bancrofti*. All participants had leg lymphedema, three included people with arm lymphedema [14, 25, 40] and two did not specify

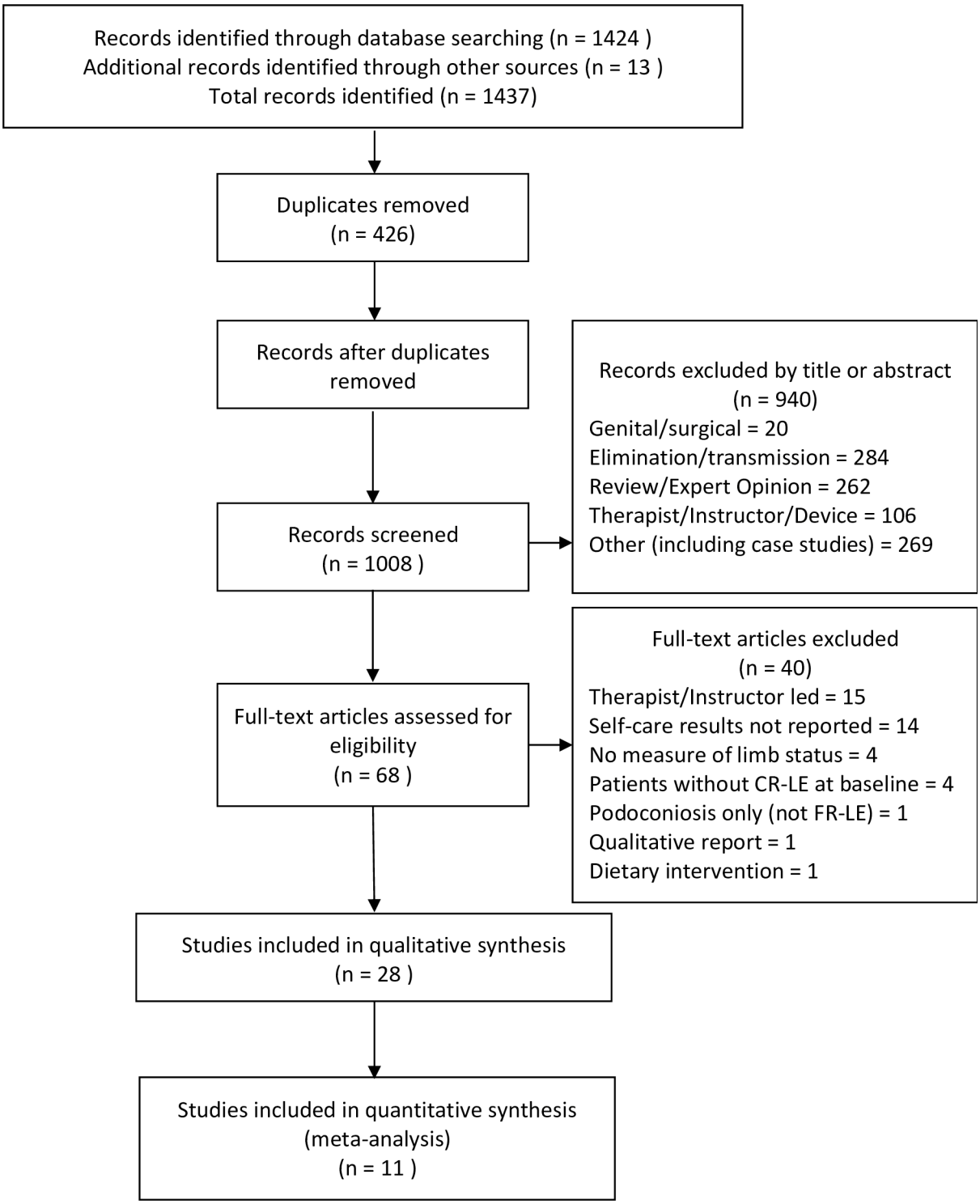

Fig 1. PRISMA flow chart of search results.

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the affected limb [33, 41]. Both genders were represented with a greater proportion of females (42.5% - 87%) and although children were admitted in 14 studies most participants were adults with a mean or median age between 35 and 57 years (range 10 to 98 years). Sample sizes ranged between 14 and 1578 with 14 studies of more than 90 participants.

In contrast to FR-LE, most participants in the ten CR-LE studies were women with arm lymphedema after breast cancer and only one RCT included males and participants with leg lymphedema [27]. Other than one Indian cohort [42] all were conducted in developed countries; sample sizes were smaller and four studies had less than 30 participants (range 18–138). Compared to FR-LE studies, the mean or median age of participants in CR-LE studies was higher, between 47 and 66 years (range 25–87 years) and none included adolescents. Population characteristics for all studies are provided in S2 Tables.

Assessment of limb status

Staging (or grade) of lymphedema was based on clinical assessment of limb size and skin changes. FR-LE studies used either the WHO criteria of Grades 1–3 [43], WHO criteria of Grades I–IV [44] or a seven stage criteria developed by Dreyer, Addiss [11]. CR-LE studies did not state the staging criteria used. Two CR-LE [29, 42] and three FR-LE studies [32, 36, 39] excluded participants with later stages of disease and participants with lymphedema stage 0 were included in two FR-LE studies [30, 36]. The water displacement method [45], which is considered the international gold standard [1], was used to calculate limb volume in both CR-LE and FR-LE studies. Only studies on CR-LE used electronic measuring devices, specifically; multi-frequency bio-impedance spectroscopy which measures extracellular fluid loads [46] and perometry which calculates limb volume using a truncated cone formula from circumferences at 3 millimetre intervals [47]. Limb circumference by tape measure was included in most studies and reported as limb volume, calculated using a truncated cone formula at four or five centimetre intervals, as raw circumference values at fixed points or as a combined average of all points. Studies on unilateral lymphedemas frequently reported on relative limb volume (difference between the affected and unaffected limbs) and percentage change in relative limb volume (RLV) over time. Methods used to assess limb status in each study are provided in S2 Tables.

Interventions

Basic self-care. Basic self-care in FR-LE studies centred on meticulous skin care including frequent washing and drying of the affected limb with soap and water, limb elevation while sleeping and during the day when possible, range of motion (ROM) exercises and application of topical creams to entry lesions. Hygiene equipment such as bowls, soap and towels were provided in nine studies [23, 32, 35, 36, 39, 40, 48–50]. Medicated topical creams and oral antibiotics or anti-inflammatories were recommended or supplied for treatment of ADLA in all prospective studies. In CR-LE studies meticulous attention to skin integrity took the form of regular use of emollients and attention to nail care rather than instruction in washing and drying. Remedial exercises and compression garments were also universally recommended.

Additional components of self-care. After basic self-care, the most studied intervention was a home based exercise program on women with CR-LE after breast cancer which included resistance exercise (using gravity or light weights to provide resistance against muscular contraction) [28, 31, 42, 51], pole walking [52], yoga [38] or deep breathing with gentle arm exercise [53]. Self-lymphatic drainage (SLD) is a form of gentle self -massage used to promote the flow of lymph and was taught or recommended in several studies [27, 29, 37, 38], one RCT compared selected essential oils blended into the massage medium to plain cream [27]. Three

RCTs on FR-LE compared medicated soap [23] or medicated cream [24, 25] to plain soap or cream in the daily self-care routine and two included one group who used antiseptic ointment daily [30, 33]. Compression therapies were recommended in all CR-LE studies, either as continued use of a previously prescribed garment [27, 31, 38, 42, 53] or a new compression sleeve supplied at study commencement [28, 29, 51, 52, 54]. One FR-LE study trained participants in self-bandaging and provided custom made garments [37] while another recommended the use of compression but reported infrequent or no use among participants [14]. No CR-LE studies utilized self-bandaging.

Instruction in self-care. Most self-care protocols were delivered to individuals and their families or carers in outpatient clinics or in-home settings and ranged from a single hour of education and demonstration [29, 32, 38] to daily training over 4 days [37]. Those studies which stipulated a published protocol for morbidity management of FR-LE followed the WHO Community Based Home Care guidelines [14, 34, 35] or the 'New Hope for People with Lymphoedema' booklet by Dreyer, Addiss [11] [32]. Six FR-LE and three CR-LE studies received printed instructions [14, 36, 37, 40, 41, 49] [28, 38, 42] and two CR-LE studies delivered home support through digital media [31, 38].

Monitoring and follow-up. Participants in most FR-LE studies attended fortnightly or monthly home or clinic visits with extra, surprise field checks in five studies [24, 25, 30, 36, 48]. Some participants were able to access a clinic or health care worker as needed [41, 50] while others recorded ADLA events in a log book [32, 49]. Participants in all prospective CR-LE studies attended measurement clinics at intervals of between two and twelve weeks. Overall FR-LE interventions were of longer duration, typically 12 months (range 4.5–36 months), five followed participants for 12 months after the intervention ceased [24, 25, 32–35] and one was a retrospective follow up on participants in a previous trial by Shenoy, Kumaraswami [25] after 12 months of unsupervised self-care [55]. Compared to FR-LE studies CR-LE studies were generally shorter (range 1–6 months) and only two studies followed subjects for 12 months [29, 54].

Effects of interventions

Basic self-care for FR-LE. *Effect of basic self-care on ADLA in FR-LE.* Eleven studies reported on this outcome (n = 2954). Episodes of ADLA were assessed in a clinic, recorded in a log book or by participant report. Before and after periods were unequal in some studies which reported baseline ADLA using patient recall of the previous 12 months and then collected data as frequently as every two weeks during the intervention.

Proportion of persons experiencing ADLA episodes. Results of eight studies were combined to estimate the effect of basic self-care on ADLA episodes (Fig 2). A random effects model was used due to a high level of heterogeneity between results ($I^2 = 96$). Overall, the proportion of participants reporting any ADLA attacks was reduced by 54% (RR = 0.46; 95% CI 0.26 to 0.82). Five studies reported significant reduction in the proportion of participants who experienced any ADLA [14, 36, 39, 40, 50] and two found non-significant reductions [41, 55]. Only the placebo drug group in an RCT reported an increase in persons experiencing any ADLA over 24 months (RR = 1.09, 95% CI 0.74, 1.63) [32]. In the pseudo-RCT which compared three service delivery methods the proportion of participants who reported no episodes in the preceding 6 months increased from 6% at baseline to 93.5% after 12 months [50].

Frequency and duration of ADLA. Seven studies reported on this outcome. Six prospective cohorts (n = 1471) found that ADLA frequency or duration reduced between 26% and 100% after 6 to 24 months of self-care [35, 39, 48, 50] and this was significant in two cohorts [14, 36]. Where ADLA incidence was reported by lymphedema stage the best improvements were among participants with stages III and IV (81.3%, p = 0.022) and stage II (80.8%, p<0.001) [14] and

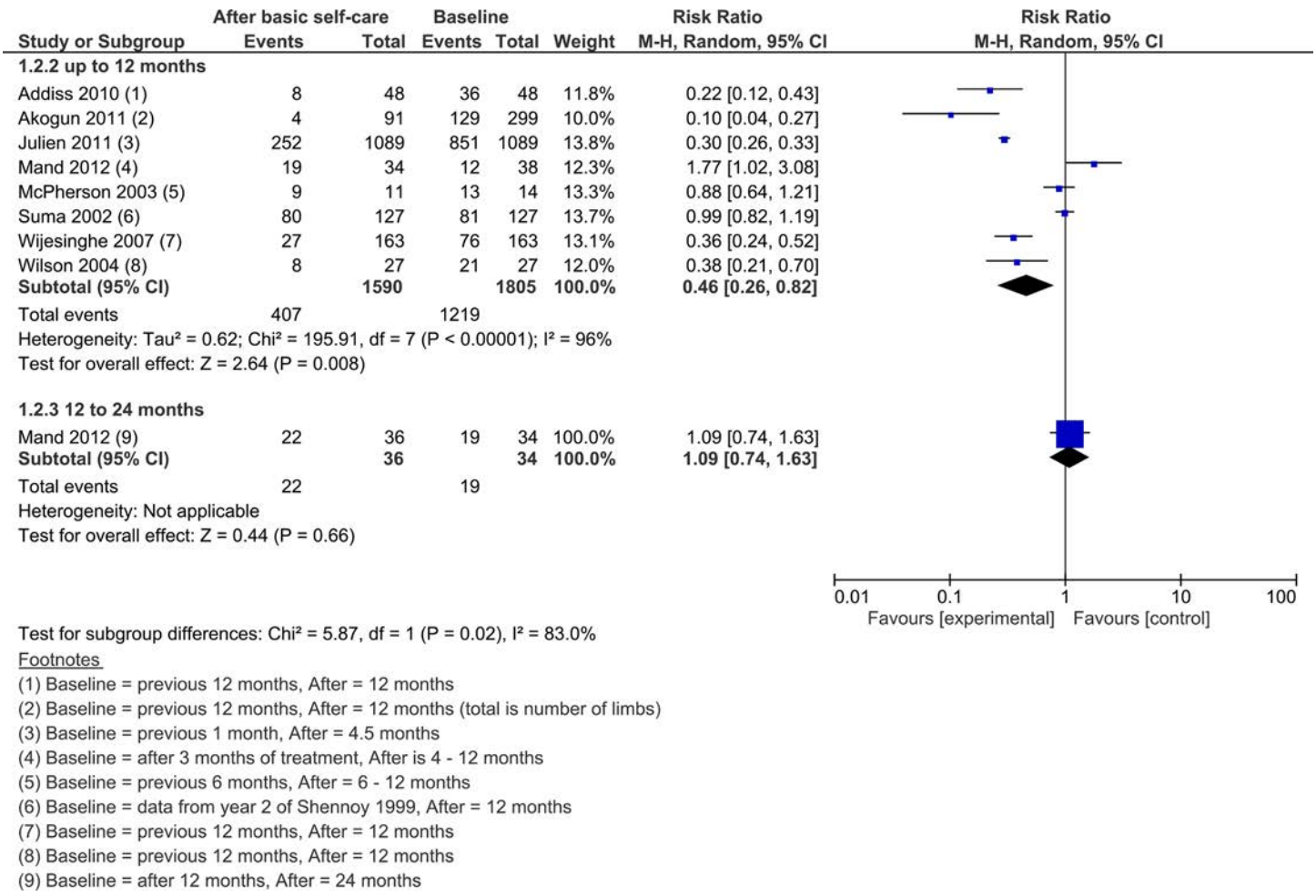

Fig 2. Forest plot of the proportion of FR-LE participants experiencing any ADLA episodes.

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more than 60% reduction was achieved in all stages after 6 months [35] and 12 months [48]. Four studies found either; a decrease in the mean duration of ADLA episodes (range 24% - 37.5%) [14, 48] or in the number of working days lost (mean 39%, range 28% - 44%) [34]. In the pseudo RCT the proportion of participants who had experienced ADLA that lasted more than 4 days in the previous year dropped by 78.6% and although 23.4% had reported ADLA that lasted seven days or more at baseline, by the end of the study this had reduced to none [50]. The retrospective follow up of participants in a previous trial by Shenoy, Kumaraswami [25] showed that some benefits were lost 12 months after monitoring ceased and mean ADLA incidence had increased by 65% [55], however this was still 40% lower than before commencement of self-care in the original RCT. The study by Mues, Deming [35] also found that although the significant reductions in ADLA achieved at 6 months (60%) were partially lost after 12 months, the rate remained lower than at baseline (35%) and this was maintained at 24 months. Results of basic self-care on frequency and duration of ADLA episodes are provided in S3 Tables.

Effect of basic self-care on FR-LE status

Seven studies (n = 1073) reported on change in either objective assessment of lymphedema stage or limb volume, or participant perception of limb status. WHO staging criteria was used

Table 2. Change in FR-LE limb volume after 12 months of self-care in Addiss et al 2010 [36].

Stage #	Baseline Mean ml (range) *	After 12 months Mean ml (range) *	N (legs)	% reduction	P
0	1610 (1080–2160)	1604 (1310–2130)	26	0.3%	
1	1937 (1515–2760)	1786 (1470–2030)	9	7.8%	
2	1986 (1450–2835)	1909 (1230–2970)	41	4.0%	p<0.05
3	2839 (1920–3700)	2354 (1580–3260)	15	17.1%	p<0.05
4	3644 (2390–4760)	3082 (1510–4010)	5	15.4%	

Dreyer et al 2002 [11]

ml = millilitres

* Water displacement method

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more frequently (four studies) than the seven stage Dreyer system (two studies). Limb volume was quantified by water displacement (three studies) or limb circumference (three studies).

Changes in limb volume. In a study which excluded participants with stages 5–7 using the Dreyer, Addiss [11] criteria, limb volume reduced in stages 0–4 after 12 months and this was significant in stages two and three which reduced by 4% and 17.1% respectively [36] (Table 2). In a cohort with unilateral leg lymphedema participants who reported a perceived reduction or no change in limb volume (54%) were more likely to have earlier stage FR-LE whereas participants who reported a perceived increase (46%) were more likely to have later stages of disease [48].

Change in lymphedema stage. Three reports on two studies (n = 533) found a significant proportion of participants had reverted to a lower stage after 12 [14] and 24 months [34, 35] of basic self-care. This effect was greater in participants with early or moderate stage FR-LE, whereas the proportion of people with more advanced disease either stayed the same [35] or increased slightly (not significant) (Table 3). Other studies reported no change in lymphedema stage [32, 39].

Effect of basic self-care on perceived disability and QOL in FR-LE

Two studies reported on this outcome using either the Dermatology Quality of Life Index (DQLI) [56] or the WHO Disability Assessment Schedule II (WHO DAS II) [57]. Both studies used the seven stage criteria for FR-LE and reported significant improvement in either; all stages after 12 months (n = 14) [41] or in stages 3–7 after 24 months (n = 370) [34]. Results for changes in perceived disability and quality of life are provided in S3 Tables.

Table 3. Proportion of Participants by FR-LE stage after 6–24 months of self-care.

Study ID	Stage	Baseline	6 months	12 months	24 months
Mues et al 2014[35] and Budge et al 2013[34][1]	1–2	48.73%	54.01%	55.76%	60.13% p = 0.0064
	3–4	37.57%	32.41%	30.22%	25.32% p = 0.0006
	5–6	12.70%	13.58%	14.02%	14.56%
Wijesinghe et al 2007[14][2]	I	8.6%	n/a	16%*	n/a
	II	52.7%	n/a	46%	n/a
	III	31.3%	n/a	31%	n/a
	IV	7.4%	n/a	8%	n/a

*Eleven people reverted from Stage II to Stage I (p = 0.012)

1 = Stages per Dreyer et al 2002 [11]

2 = Stages per WHO 2003 [13]

doi:10.1371/journal.pntd.0004740.t003

Basic self-care for CR-LE

No study assessed the effect of self-care alone on CR-LE.

Self-care using topical medication for FR-LE

Five studies (n = 460) used medicated creams or soap in the daily self-care protocol. Three RCTs were used to compare either medicated soap [23] or medicated cream [24, 25] to plain soap or plain cream and medicated ointment was used daily by one self-care group in two other trials [30] [33]. All interventions were of 12 months duration and three studies followed participants for a further year after the intervention ceased [24, 25, 33].

Effect of self-care with topical medication on frequency and duration of ADLA

Neither of the RCTs which compared medicated cream to plain cream found any between group differences. After the 12-month intervention a significant reduction in annual ADLA episodes of between 63.83% [25] and 77.57% [24] was recorded by all groups and at 24 months the mean annual incidence was still significantly lower than at baseline by 59.52% and 65.02% respectively. During the follow up year, annual incidence in the groups who had used plain cream during the intervention continued to reduce, whereas both groups who had used antibiotic cream experienced an increase (not significant). The trial which compared antibiotic soap to plain soap also found no difference between groups and reported results as for a single cohort [23]. This cohort and the medicated cream groups in two trials (n = 340) all reported significant reductions in mean ADLA episodes of between 62.5% and 65.6% after 12 months [23, 30, 33]. One group was followed for 12 months after the intervention and an overall reduction of 73% from baseline was recorded [33]. Results of the effect of medicated cream or soap on ADLA are shown in S3 Tables.

Effect of self-care with topical medication on FR-LE status

Two trials reported on this outcome (n = 120). In a 12-month intervention on unilateral leg lymphedema [30], raw circumference values for the affected limb reduced between 27.6% - 92% with the greatest reduction at the calf of participants with Grade 2 FR-LE (WHO grades 1–3 [43]) and the least reduction at the ankle in participants with Grade 3 (Fig 3). In this trial the difference in circumference between affected and unaffected limbs also reduced significantly at all time points. In the trial which used water displacement to measure limb volume at baseline and then again during ADLA [24], limb volume increased during 80% of episodes and remained elevated in 73% of cases after two weeks.

Self-care using topical medication for CR-LE

No CR-LE studies investigated the addition of medicated creams or soaps.

Home based exercise for FR-LE

No studies assessed the effect of prescribed exercises on FR-LE.

Home based exercise for CR-LE

Seven studies assessed exercise interventions of between eight weeks and six months duration. All participants were women with unilateral arm lymphedema after breast cancer (n = 197) and six studies reported significant benefits in at least one outcome.

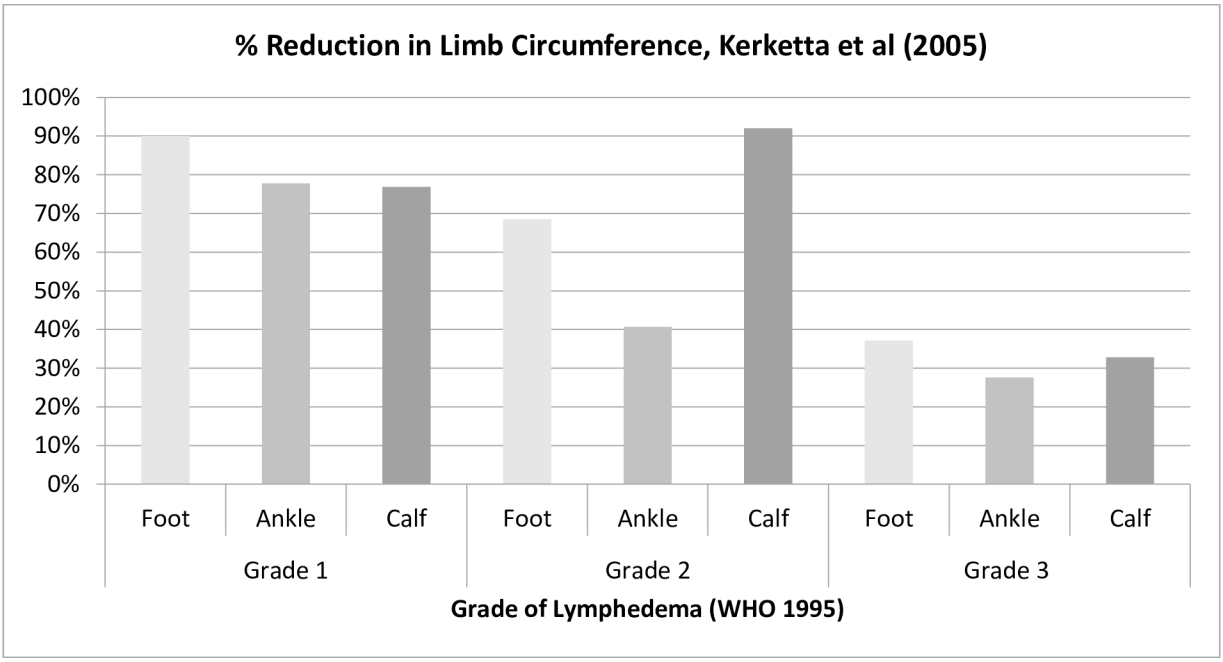

Fig 3. Change in FR-LE limb circumference after 12 months of medicated cream use.

doi:10.1371/journal.pntd.0004740.g003

Effect of home based exercise on CR-LE status

Change in limb volume. Results of three studies that reported on change in relative limb volume (RLV) (difference between the affected and unaffected limbs) were combined to estimate the effect of home exercise (Fig 4, n = 54). A random effects model was used due to a high level of heterogeneity between results ($I^2 = 0$). Overall, RLV reduced by 1.31% (95% CI -4.73, 2.11).

Statistically significant reduction in limb volume was recorded 10 minutes after commencing a deep breathing exercise with gentle arm movements [53], after eight weeks of pole walking [52] or isotonic arm exercises with deep breathing [42], after ten weeks of weight lifting [51] and in both groups after 12 weeks of either gravity resisted exercise or self-care with hand pumping [28]. In the latter trial both groups had reduced further after six months but this was significant only in the gravity resisted exercise group. This trend was supported by a reduction in arm volume in the home based exercise group in an eight week trial [31] and in a group of women who practiced yoga at home for six months after an initial four week intervention [38]

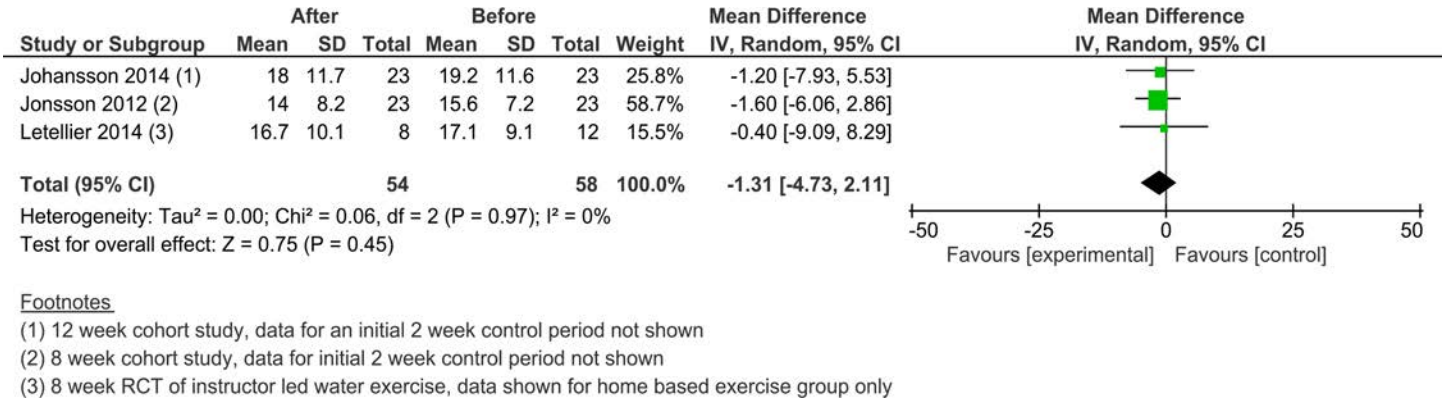

Fig 4. Forest plot of percentage change in relative CR-LE limb volume after exercise.

doi:10.1371/journal.pntd.0004740.g004

whereas the participants who discontinued yoga practice had experienced an increase (not significant). Results of the effect of home based exercise on limb volume are given in S4 Tables.

Effect of home based exercise on limb function, self-reported symptoms and QoL

Seven studies (n = 295) reported improvement in one or more of these outcomes.

Change in limb function. Significant improvements were found in grip strength [31], muscle strength [51] and cardiovascular fitness [52] but not in ROM [28]. Three studies reported an improvement in participant perception of limb function using various versions of the Disabilities of the Arm, Shoulder and Hand (DASH) questionnaire [58] and this was significant in the home exercise group in one RCT [31].

Change in self-reported symptoms. Likert scales and visual analogue scales were used to assess participant perception of symptoms in the affected limb in four studies (n = 143). Significant improvement was found in; pain, heaviness, tightness, pins and needles, and perception of limb size after one month of deep breathing with gentle arm exercise arm [53] and in arm tightness after eight weeks of pole walking [52].

Change in quality of life. Two studies on home based exercise reported significant improvement in all domains of the Short Form 36 Health questionnaire after eight weeks [42] and in the Functional Assessment of Cancer Therapy–Breast (FACT-B) after 12 weeks [31].

Self-lymphatic drainage (SLD) and compression therapies for FR-LE

Only one FR-LE study emphasized the use of SLD and instructed participants in daily self-bandaging. After three weeks of self-bandaging they were fitted with compression garments which were then worn daily for the remainder of the study [37]. Although the self-care protocol in the study by Wijesinghe, Wickremasinghe [14] recommended compression it was not routinely used and results of that study were reported in the basic self-care section.

Effect of SLD and compression on FR-LE status

Change in limb volume. **S**ignificant reductions were recorded after three, six and nine months of performing comprehensive self-treatment including compression therapy (n = 33 legs) [37]. Results for this study are provided in S3 Tables.

Self-lymphatic drainage (SLD) and compression therapies for CR-LE

One study on both arm and leg lymphedema [27] and two studies on arm lymphedema [29, 54] included SLD in the self-care program (n = 121). The RCT with the mixed arm and leg population investigated the benefits of using Aromatherapy (essential oils) in the massage medium compared to plain cream but found no significant difference between groups and reported most results as a single cohort using SLD [27]. No CR-LE studies included self-bandaging but regular use of a compression garment was recommended in all studies. New garments were supplied at commencement in four studies [28, 29] of which two [51, 52] allowed a control period before the intervention to adjust for the effect of the garment.

Effect of SLD and compression on CR-LE status

Change in limb volume. Two studies that used SLD with compression garments [27, 54] and one group of women that had mild arm lymphedema and used SLD without compression [29] reported significant reduction over three and six months of between 2.59 and 60% (Table 4, n = 121). In the six-month trial of SLD on people with both arm and leg lymphedema,

Table 4. Change in CR-LE limb volume (ml) after 3–6 months of SLD.

Study ID	Limb |Duration (months)|		Before Intervention		After Intervention		% Reduction (95% CI)
	Limb		Volume ml	N	Volume ml	N	
Anderson et al 2000[29]	Upper limb (RLV)[2] Median (range)	3	361 (78–1184)	22	n/a	22	60% (43% - 78%)
Barclay et al 2006[27]	Upper limb (RLV)[1] Median (range)	6	107.0 (-372.0–2421.0)	81	60.0 (-334–2344)	71	43.92%
Barclay et al 2006[27]	Right lower limb (WLV)[1] Mean (SD)	6	6218.3 (1772.5)	81	6057.1 (2093.0)	71	2.59%
Barclay et al 2006[27]	Left lower limb (WLV)[1] Mean (SD)	6	6177.9 (1857.9)	81	5797.9(2014.9)	71	3.20%
Koul et al 2007[54]	Upper limb (WLV)[1] Mean	3	2685	18	2587	18	24% p<0.0001

WLV = Whole limb volume

RLV = Relative limb volume, difference between affected and unaffected limbs

1 = Volume calculated from limb circumference at 4cm intervals

2 = Volume calculated from limb circumference at 5cm intervals

SD = standard deviation

n/a = data not provided

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significantly more participants experienced an improvement in limb volume than got worse [27].

Effect of SLD and compression on self-reported symptoms and wellness in CR-LE

The Measure Yourself Outcome Profile 2 [59] was used to assess quality of life in participants performing SLD with or without Aromatherapy and both groups reported significant improvements at all time points up to 6 months [27].

Discussion

In reviewing the evidence for self-care in FR-LE and CR-LE, marked differences were apparent both between and within settings and some key opportunities for improvements were identified. Evidence from the FR-LE population showed that basic self-care alone is effective in preventing ADLA which is consistent with results of a recent review of the effect of hygiene based interventions on FR-LE [60]. Hygiene alone may halt disease progression but is less likely to reduce limb volume and evidence from the CR-LE population indicated that greater volume reductions are achieved when activities such as progressive resistance exercise are included in the self-care routine. Whilst basic self-care was the primary intervention in almost 60% of studies on FR-LE, no CR-LE studies assessed basic self-care alone; rather the self-care group when included were always as controls. Best practice guidelines in CR-LE management are still dependent on therapist performed interventions; however evidence from the FR-LE population suggests that more effort to involve CR-LE patients in their own self-treatment may relieve the financial burden of therapist based care in this population.

The exclusion of any group in comparative studies that received drug or therapist based interventions meant that although ten RCTs were reviewed the bulk of evidence came from observations of a single cohort in studies rated of moderate or weak methodological quality where only one or two groups were performing self-care. There was also inconsistency between assessment techniques and reporting methods. Therefore a review which includes all interventions for FR-LE and CR-LE may provide data for more rigorous meta-analysis. None the less, this first review to systematically examine the similarities and differences in self-care for

CR-LE and FR-LE has opened a pathway for further investigation of transferrable strategies for lymphedema management in disparate settings.

The available resources in each study setting were reflected in the simplicity or complexity of devices used to measure change in limb status. All CR-LE studies used water displacement, bio impedance spectroscopy, perometry or a truncated cone formula at small intervals to quantify limb volumes. These methods can detect very small changes which, although statistically significant, might be of minimal clinical significance. In contrast most FR-LE studies relied on less precise measures and less than one quarter used either water displacement or a truncated cone formula. A further 18% of studies used three or four fixed circumference points to compare affected and unaffected limbs or summed or averaged these measures. These methods, especially summed or averaged circumferences lack the precision to detect small changes in limb volume and this could account for variations in reported outcomes between the CR-LE and FR-LE groups. More frequently, FR-LE studies relied on assessment of lymphedema by stage and the use of criteria with only three or four groups was common, since even studies which used the seven stage criteria often grouped them into early, moderate or late stage disease. These graduations may lack the precision to detect small changes and participants who changed from a higher to a lower stage or vice versa may not have always been detected, thereby under or over estimating the effectiveness of the intervention. Most studies tried to minimise inter- or intra-observer variation in staging but the subjective nature of these assessments may also have contributed to the disparate results. These limitations may explain why some studies reported reduction in limb volume without a corresponding change in lymphedema grade. Overall studies which used more precise measuring protocols more often reported significant evidence for volume reduction in both settings.

Less than 12% of FR-LE studies investigated the effect of self-care on subjective symptoms or functional deficits whereas this was reported in 70% of CR-LE studies. The evidence for improvement was weak, mainly due to the disparate range of measuring tools employed, but the overall trend was that a reduction in limb volume was accompanied by improvements in symptoms, perceived disability, overall wellbeing and quality of life. This suggests that self-reported parameters could be used as a proxy for objective measures when these are not available and provide valuable information about the lived experience of FR-LE.

Oral or topical medications for ADLA were used in almost all FR-LE studies but the influence of these could not be adequately separated from the effect of other components of self-care and it is unclear what bearing this had on the results. Although groups treated specifically with oral antibiotic or deworming medications were excluded from the data synthesis some reports showed that the placebo drug groups performing basic self-care had better long term results than groups that had initially received oral medications [24, 25, 33]. Studies on topical medications for ADLA showed no additional benefit over self-care using placebo creams or soaps and the necessity of prophylactic oral medication for ADLA remains controversial. Similarly, in CR-LE studies the use of a compression sleeve was considered an integral aspect of self-care but few studies controlled for the effect of a new garment or included frequency of garment use in the statistical analysis. Where this was done the effect of the compression sleeve was shown to be significantly correlated with improvement in all groups. Whilst these treatments might be considered to be core elements of self-care, access to medication in poorly resourced settings and non-adherence to compression therapies in CR-LE warrant the investigation of self-care protocols that do not rely on these components.

Effective self-care implementation requires some degree of education, instruction or demonstration and the role of the educated health worker or trained volunteer cannot be ignored. FR-LE studies which provided frequent monitoring and support were associated with greater improvements than studies which offered minimal or no support services. The study by Suma,

Shenoy [55] which retrospectively reviewed participants in a previous drug based RCT indicated that without monitoring, program effectiveness is lost over time, an effect also found in a later follow up of the study by Addiss, Louis-Charles [36] which could not be included in this review [61]. Ultimately, the long term success of any self-care intervention will depend on individual ownership of and adherence to the daily self-care practices and the level of family or local support available. This was demonstrated clearly in the study by Akogun and Badaki [50] where one group was able to alter the program design to suit their immediate cultural and social constraints and reported good outcomes, whereas the two groups who could not alter the program design to suit their personal circumstances experienced a large loss to follow up.

Despite the wide variation in measurement techniques and support services, it was apparent that hygiene-centred, basic self-care can reduce the frequency and duration of ADLA episodes by approximately 50%, and this was particularly beneficial for people with later stages of disease. There was less evidence for a reduction in limb volume but the study by Joseph, Mony [24] showed that ADLA led to an increased limb volume which persisted after the infection had been treated and Mues, Deming [35] demonstrated that ADLA is related to days of work lost. Thus a reduction in ADLA episodes without change in limb size may still improve outcomes for many individuals. This was illustrated histologically in the study by Wilson, Guarner [39] which showed that basic self-care improved skin integrity and prevented new infections while limb stage remained the same. Where a reduction in limb volume was reported in FR-LE, greater benefits were experienced among participants with early stages, suggesting that implementation of a self-care routine as soon as lymphedema is detected has the potential to curtail the number of cases that progress to advanced stages. Current guidelines for FR-LE will not assist program managers to find and address these earliest stages of lymphedema yet this may be the optimal time to intervene in terms of volume reduction and long term prevention of ADLA. Basic hygiene is enough to control ADLA but this review has shown that limb volume is more difficult to reduce in later stages regardless of the setting [27, 48]. Advanced lymphedema is characterised by fibrosis and fatty induration of the tissues which become much more difficult to reduce than in early stages where the swelling is more characteristically due to protein rich fluid. So to reduce limb volume more intervention is required at an earlier stage than is currently indicated in the WHO guidelines.

Reduction in limb volume was reported in all CR-LE studies, all of which included at least one additional component of self-care. Since publication of the 2005 study by Moseley, Piller [53], specific deep breathing exercises appear frequently in interventions for CR-LE as was evidenced by their inclusion in six studies in this review. Home based exercise, including deep breathing, is easy to perform, require no financial resources, can be continued alone after minimal initial instruction and may contribute to overall improvement in health and wellbeing, but exercise advice included in the current protocols for FR-LE is very limited. Simple resistance exercise and deep breathing could be easily incorporated into CBHC particularly in cultures where activities such as Yoga or Tai Chi may be readily available and acceptable and the addition of such components to current WHO recommendations warrants investigation.

In FR-LE, issues pertaining to infection, wet environments and lack of foot wear make compression therapies problematic. However, Bernhard, Bernhard [37] and other studies that could not be included in this review [62] have showed that people with FR-LE are capable of performing complex compression bandaging and daily use of compression garments. This requires a greater initial investment in the training and education of people with FR-LE so that they have a better understanding of the purpose and effect of each treatment component. The long term benefit of this increased investment was demonstrated in the study by Bernhard, Bernhard [37] where limb volume reduction was significantly greater in the self-treating group compared to the therapist treated group (results for the therapist treated group were not

included in this review). This supports consideration of compression therapies in FR-LE when possible and although the evidence is limited, also suggests that self-bandaging could be further explored for people with CR-LE.

Although people with arm lymphedema were included in several FR-LE studies, results were not reported by limb. Only the CR-LE study by Barclay, Vestey [27] reported results by affected limb and in this study the impact of SLD on volume reduction was much greater for arms than legs. This limited comparison of results by limb implies that interventions may deliver different results depending on the location of the lymphedema. Similarly, some studies allowed participation of children as young as five years old but no study gave an analysis by age to determine if children had better or worse outcomes than the adult subjects, nor was the effect of gender explored. It is possible that age and gender influence self-treatment outcomes and investigation of different components of self-care by limb, age and gender should be considered.

Since disability from existing LF will continue to increase for several decades even after transmission has been successfully interrupted, and increasing cancer survivorship is a primary focus of cancer research, it is probable that the incidence of new lymphedema cases from both causes will continue to increase for the foreseeable future. Focussing efforts toward greater emphasis on early intervention and prevention has the potential to alleviate this future burden. Implementation of morbidity management in the global effort to eliminate LF requires evidence based strategies to attract and maintain funding, and reducing the burden of CR-LE for all cancer survivors requires more research about lymphedema of the leg. In both cases high quality studies that investigate reversal of early stage disease and analysis of individual components of self-care by age, gender, stage and location of lymphedema are essential to determining optimal, financially sustainable, management.

Supporting Information

S1 Tables. Inclusion criteria and methodological quality of assessed papers.
(DOCX)

S2 Tables. Population characteristics of all studies.
(DOCX)

S3 Tables. Effect of interventions on Filariasis Related Lymphedema (FR-LE).
(DOCX)

S4 Tables. Effect of interventions on Cancer Related Lymphedema (CR-LE).
(DOCX)

Author Contributions

Conceived and designed the experiments: JD SG. Performed the experiments: JD SG. Analyzed the data: JD. Contributed reagents/materials/analysis tools: JD PG. Wrote the paper: JD SG PG.

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S1 Tables: Inclusion Criteria and Methodological Quality of Assessed Papers

Table S1.1: Inclusion and Exclusion criteria as registered on the PROSPERO database

Category	Inclusion Criteria	Exclusion Criteria
Participants	Participants living in developing countries with lymphedema secondary to infection with lymphatic filariasis. Participants living in developed countries with lymphedema secondary to treatment for cancer.	Participants who have genital only lymphedema without involvement of at least one limb. Participants who have previously received surgical interventions as part of lymphedema management.
Intervention	Any intervention or combination of interventions given for the treatment of lymphedema that can be performed by the participant or a family member. Any intervention of combination of interventions given to address factors contributing to progression of lymphedema that can be performed by the participant or a family member.	Mass drug administration studies in filariasis endemic areas where lymphedema outcomes are not specifically measured. Studies using benzo-pyrones or other drug treatments which are no longer in use. Surgical interventions for hydrocele or other surgical interventions for lymphedema. Studies involving treatment dependent on lymphedema specialist therapist services or instructor led programs. Studies involving treatment use of specialist lymphedema treatment devices such as compression pumps or custom made garments. Drug trials where data for a self-care only (placebo) group are not given. Interventions which address genital lymphedema only and do not provide management for at least one limb.
Types of studies	Any peer reviewed publication assessing a self-care intervention for secondary lymphedema where pre and post test data evaluating physical changes of the limb has been collected and reported.	Reviews/opinion/editorial articles. Single case studies (except as part of a cohort study). Economic evaluations or qualitative studies that do not include pre and post test measurement of lymphedema status.

Table S1.2: Methodological Quality of papers assessed using the CASP RCT Appraisal Form

Author (Year)	Study Rating	Did the trial address a clearly focussed issue	Was the assignment of patients to treatment randomized	Were all of the participant who entered the trial accounted for at its conclusion	Were patients, health workers and study personnel blind to the treatment	Were the groups similar at the start of the trial	Aside from the experimental intervention were the groups treated	Can the results be applied to the local population	Were all clinically important outcomes considered	Are the benefits worth the harms and costs	**Yes Score / 9 questions**
Addiss et al 2011	**W**	Y	Y	N	CT	Y	CT	Y	N	Y	**5**
Akogun & Badaki 2011	**W**	Y	N	N	N	CT	N	Y	Y	Y	**4**
Andersen et al 2000	**M**	Y	Y	Y	CT	N	Y	Y	Y	Y	**7**
Barclay et al 2006	**M**	Y	Y	Y	N	CT	Y	Y	CT	Y	**6**
Joseph et al 2004	**S**	Y	Y	Y	Y	Y	Y	Y	Y	Y	**9**
Jeffs et al 2013	**W**	Y	Y	Y	N	Y	Y	N	Y	Y	**7**
Kerketta et al 2005	**S**	Y	Y	Y	CT	CT	Y	Y	Y	Y	**7**
Letellier et al 2014	**M**	Y	Y	Y	CT	N	Y	Y	Y	Y	**7**
Mand et al 2012	**M**	Y	Y	Y	CT	Y	N	Y	Y	Y	**7**
Shennoy et al 1998	**M**	Y	Y	Y	CT	CT	CT	Y	N	Y	**5**
Shennoy et al 1999	**W**	Y	Y	Y	CT	N	Y	Y	N	Y	**6**

S=Strong, M=Moderate, W=Weak
Y=Yes, N=No, CT=Can't Tell

Table S1.3: Methodological Quality of papers assessed using the CASP Cohort Appraisal Form

Author (Year)	Study Rating	Did the study address a clearly focussed issue	Did the authors use the appropriate method to answer the question	Was the cohort recruited in an acceptable way	Was the exposure adequately measured to minimize bias	Was the outcome accurately measured to minimize bias	Have the authors identified all important confounding factors	Have they taken account for the confounding factors in the design or	Was the follow of subjects complete enough	Was the follow up of subjects long enough	Do you believe the results	Can the results be applied to the local population	Do the results of this study fit with other available evidence	Yes Score / 12 questions
Addiss 2010	**M**	Y	Y	N	Y	CT	Y	Y	CT	Y	CT	Y	Y	**8**
Bernhard 2003	**M**	Y	Y	CT	Y	Y	N	CT	Y	Y	Y	CT	Y	**8**
Budge 2013	**W**	Y	Y	Y	N	Y	CT	Y	N	Y	Y	Y	CT	**8**
Das 2013	**W**	Y	Y	CT	Y	N	N	N	CT	Y	CT	Y	Y	**6**
Douglass 2012	**M**	Y	Y	Y	CT	Y	CT	N	CT	Y	Y	Y	Y	**8**
Gautam 2011	**W**	Y	N	CT	Y	Y	N	CT	CT	CT	Y	Y	Y	**6**
Johansson 2014	**W**	Y	Y	Y	Y	N	CT	N	Y	Y	CT	Y	Y	**8**
Jonsson 2014	**W**	Y	Y	Y	Y	CT	CT	N	N	Y	Y	Y	Y	**8**
Jullien 2011	**W**	Y	Y	Y	N	CT	N	Y	N	Y	Y	Y	Y	**8**
Koul 2007	**W**	Y	Y	Y	CT	N	N	CT	N	Y	Y	Y	Y	**7**
Mathieu 2013	**W**	Y	CT	CT	N	Y	N	N	CT	Y	CT	N	N	**3**
McPherson 2003	**W**	Y	Y	N	CT	CT	CT	CT	N	Y	Y	Y	Y	**7**
Moseley 2005	**W**	Y	N	CT	Y	N	N	Y	Y	Y	Y	Y	Y	**8**
Mues 2015	**M**	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	**11**
Suma 2002	**W**	Y	Y	Y	Y	CT	N	N	Y	Y	Y	Y	Y	**9**
Wijesinghe 2007	**W**	Y	Y	Y	N	CT	Y	N	N	Y	CT	Y	Y	**7**
Wilson 2004	**M**	Y	Y	CT	CT	Y	CT	Y	N	Y	Y	Y	Y	**8**

S=Strong, M=Moderate, W=Weak
Y=Yes, N=No, CT=Can't Tell

S2 Tables: Population Characteristics of All Studies

Table S2.1: Description of Randomized Controlled trials (RCT) on FR-LE

Study		Population		Intervention		Outcomes
Author (Year) **Country** **Setting**	**Design** **Duration and Follow Up** **Groups in Review**	**Affected limb (s)** **Inclusion criteria** **# significant baseline difference**	**N (% retention)** **Age** **Gender**	**Self-Care Program** **Equipment supplied (if any)** **Comparator groups**	**Monitoring** **Measurement Interval**	**Outcome (Outcome measure)** ***significant benefits reported**
Mand et al (2012) Ghana 21 communities	RCT, 3 groups Follow up : 24 months Group 3, n = 54	Leg (s) Stages 1-5 Minimum body weight 40kg Aged 18-60 years	n = 162 (73.5%) Mean 47.7 years (SD 10.8) Female = 71.4%	Foot hygiene: Trained using "New Hope for People with Lymphedema" (Dreyer et al 2002) Soap, towels and bowls provided Daily medication for 6 weeks : Group 1 = Amoxicillin Group 2 = Doxycycline Group 3 = Placebo	Home visit : 3, 12 and 24 months Measured : Baseline, 3, 12 and 24 months	Lymphedema Stage (Examination, Dreyer et al 2002) Limb circumference (Combined average of 4 points) ADLA frequency (Journal record) Skin thickness (Ultrasound) Endothelial Growth Factors (ELISA)
Addiss et al (2011) Haiti Hospital Clinic	RCT, 2 groups Intervention: 12 months Group 1, n= 100 Group 2, n = 100	Leg (s) Competent in self-care Reside within 10km radius of the clinic	n = 200 (98.5%) Mean 37 years (Range 11-77) Female = 87%	Hygiene and skin care: Wash legs daily, apply antifungal and antiseptic creams as needed Soap and creams provided Group 1 = Antibacterial soap Group 2 = Plain soap	Home visits: Monthly Measured at: Baseline and 12 months	ADLA frequency* (Recall previous 12 months) Compliance to self-care and use of topical creams (Self-report) Outcomes not reported by group
Akogun & Badaki (2011) Nigeria 3 local government areas	Pseudo RCT, 3 groups Intervention: 12 months Group 1, n = 131 Group 2, n = 91 Group 3, n = 103	Leg (s) Previous ADLA # group size, gender, age	n = 325 (30.2%) ≤19 years = 0.6% 20 - 49 years = 60% ≥50 years = 39.4% Female = 67.1%	Basic hygiene: Education in limb care and exercise, apply antifungal and antiseptic creams as needed Equipment and creams provided Group 1 = Community service delivery model, able to alter delivery design Group 2 = Group leader service delivery model Group 3 = Health centre service delivery model	Monthly impact monitoring Measured; Baseline, 6 and 12 months	ADLA frequency and duration (Recall previous 12 months) Limb status* (Warts, size, folds, odours, lesions)

Study		Population		Intervention		Outcomes
Author (Year) **Country** **Setting**	**Design** **Duration and Follow Up** **Groups in Review**	**Affected limb (s)** **Inclusion criteria** **# significant baseline difference**	**N (% retention)** **Age** **Gender**	**Self-Care Program** **Equipment supplied (if any)** **Comparator groups**	**Monitoring** **Measurement Interval**	**Outcome (Outcome measure)** ***significant benefits reported**
Kerketta et al (2005) India 8 villages	RCT, 3 groups Intervention : 12 months Group 3, n=100	Unilateral leg ≥ 1 ALDA previous 12 months Participants with ADLA treated and then excluded	n = 300 (84.7%) 14 - 65 years Gender not given	Foot-care: Demonstration, regular limb cleaning, keep dry, clip nails, apply ointment between toes & sides of feet, attend to injuries Tablets and cream provided Medication, twice daily for 12 days every three months : Group 1 = DEC Group 2 = Penicillin Group 3 = Antiseptic ointment (betadine)	Fortnightly clinic visits Surprise field checks Measured between 4pm – 8pm at : Baseline, 90, 180 and 360 days	Limb circumference* (3 points) ADLA frequency* (Recall previous 12 months) (Recall previous fortnight)
Joseph et al (2004) India 22 villages	RCT, 5 groups Intervention : 12 months Follow up : 12 months Group 1, n = 30 Group 2, n = 30	Leg (s) Arm (s) ≥2 ADLA previous 12 months ≥ 15 years old > 30 kg Stratified by Grade (WHO 1992)	n = 150 (90%) Mean years (SD) Group 1, 49.8 (12.5) Group 2, 49.9 (11.1) Female = 75%	Basic hygiene : Intensive training, clipping nails, nightly cleansing, keep dry, 6% salicylic-acid ointment between toes and sides of feet Tablets and cream provided Daily medication : Group 1 = Placebo + plain ointment (zinc oxide) Group 2 = Placebo + antibiotic cream (1.5% framycetin) Group 3 = DEC + plain ointment (zinc oxide) Group 4 = Penicillin + plain ointment (zinc oxide) Group 5 = DEC + Penicillin + plain ointment (zinc oxide)	Home visits every 3- 4 days Attend clinic during ADLA Measured at : Baseline, 12 and 24 months	Limb Volume (Water displacement) ADLA frequency*, severity and duration (Observed in clinic) Blood Serology during ADLA (Micro titration) Skin lesion microbiology (Cultured from swabs)
Shenoy et al (1999) India Hospital Clinic	RCT, 5 groups Intervention : 12 months Follow up : 12 months Group 4, n = 30 Group 5, n = 30	Leg (s) Arm (s) ≥2 ADLA previous 12 months	n=150 (95%) Mean 43 years (Range 18 - 67) Female = 54%	Washing Program : Nightly washing, keep dry, clip nails, apply ointment between toes and sides of feet, apply cream during infection or injury Tablets and cream provided Daily medication : Group 1 = Penicillin + plain cream (zinc oxide) Group 2 = DEC + plain cream (zinc oxide) Group 3 = DEC + Penicillin + plain cream (zinc oxide) Group 4 = Placebo + antibiotic ointment (framycetin) Group 5 = Placebo + plain cream (zinc oxide)	Attend clinic : Fortnightly or during ADLA Surprise field visits Measured at : Baseline, 12 and 24 months	ADLA by grade of lymphedema* (Examination, WHO 1992) ADLA frequency, duration* (Recall previous 12 months) (Assessed fortnightly)

Study		Population		Intervention		Outcomes
Author (Year) **Country** **Setting**	**Design** **Duration and Follow Up** **Groups in Review**	**Affected limb (s)** **Inclusion criteria** **# significant baseline difference**	**N (% retention)** **Age** **Gender**	**Self-Care Program** **Equipment supplied (if any)** **Comparator groups**	**Monitoring** **Measurement Interval**	**Outcome (Outcome measure)** ***significant benefits reported**
Shenoy et al (1998) India Hospital clinic and surrounding area	RCT, 3 groups Intervention: 12 months Follow up: 12 months Group 3, n = 40	Limb not specified Enrolled during ADLA, admitted and treated with oral antibiotics before baseline ≥2 ADLA previous 12 months Grade 0-III (WHO 1992)	n = 120 (94%) Median 40 years (Range 18 – 65) Female = 42.5%	Local limb care: Wash and dry twice daily, apply antibiotic and antifungal ointments, oral antibiotics and paracetamol during episodes Tablets and creams provided Monthly medication, single dose: Group 1 = Ivermectin Group 2 = DEC Group 3 = Placebo	Report to clinic: Fortnightly or during ADLA Measured at: Baseline, 12 and 24 months	ADLA frequency* (Recall previous 12 months) (Assessed fortnightly)

#= Statistically significant differences between groups at baseline
*= Statistically significant improvement reported for this outcome
ADLA = Acute dermato lymphangio adenitis
DEC = Diethylcarbamazine
WHO = World Health Organization
ELISA = Enzyme-linked Immunoabsorbent Assay
SD = standard deviation

Table S2.2: Description of Cohort Studies on FR-LE

Study		Population		Intervention		Outcomes
Author (Year) **Country** **Setting**	**Design** **Duration and Follow Up** **Groups in Review**	**Affected limb (s)** **Inclusion criteria** **# significant baseline difference**	**N (% retention)** **Age** **Gender**	**Self-Care Program and equipment supplied (if any)** **Comparator groups (if any)**	**Monitoring** **Measurement Interval**	**Outcome** **(Outcome measure)** ***significant benefits reported**
Mues et al (2015) India 30 villages*	Prospective Uncontrolled Intervention: 24 months	Leg (s) ≥ 3 months swelling ≥ 14 years old	n = 370 (85.1%) Mean 57 years (SD 13.94) Female = 58.92%	Basic lymphedema management: Daily washing with soap, daily exercise and elevation and wear footwear outside the home. Topical and oral treatment for infection Soap and antifungal cream supplied for 6 months	NGO program delivering community based home care (CBHC) Measured at: Baseline, 1, 2, 3, 6, 12, 18 and 24 months	Lymphedema Stage* (Grouped 1-3, 4-7 Dreyer 2002) ADLA frequency and duration (Recall previous 30 days)
Budge et al (2013) India 30 villages* same cohort as Budge et al 2013	Prospective Uncontrolled Intervention: 24 months	Leg (s) ≥ 3 months swelling ≥ 14 years old	n = 370 (85.1%) Mean 57.15 years Female = 58.92%	Home Based Care: Regular limb washing, appropriate exercise, elevation, treat bacterial and fungal infections, use footwear	NGO program delivering community based home care (CBHC) Measured at: Baseline, 1, 2, 3, 6, 12 and 24 months	Lymphedema Stage* (Grouped 1-2, 3, 4-7 Dreyer 2002) ADLA (Recall previous 30 days) Perceived Disability* (WHODASII) Lost days of work* (Recall previous 30 days)
Das et al (2013) India Research Centre	Prospective Within subject control Intervention: 12 months	Unilateral Leg Stratified by Grade (WHO 1992) 15 – 60 years	n = 97 (87%) Mean years (SD) I = 37.6 (11.2) II = 47.3 (11.1) III&IV = 52.3 (11.8) Gender not reported	Domiciliary limb hygiene: Trained in twice daily washing and drying, identify entry lesions, apply antifungal creams, oral medication for ADLA, use footwear Hygiene kit supplied monthly Affected vs unaffected limb volume	Surprise checks Measured at: Baseline, 3, 6 and 12 months	Limb volume (Water displacement) ADLA Frequency and duration (Recall previous 12 months (Observed) Locomotor function (Indian Government Guidelines)
Mathieu et al (2013) Togo 7 endemic areas	Prospective Uncontrolled Intervention: 3 years Representative sample of the National program	Leg (s) Patients with log books available for analysis	n = 341 (55.13%) Mean 48 years (Range 12 - 98) Female = 61%	Footcare: Trained in regular washing and drying, elevation, exercise Soap, towels and illustrated booklet provided	Log book Home visits: Twice in the first week, weekly then monthly Log book reviewed every 3 months	ADLA frequency (Log book) Program adherence* (Log book)

Study		Population		Intervention		Outcomes
Author (Year) **Country** **Setting**	**Design** **Duration and Follow Up** **Groups in Review**	**Affected limb (s)** **Inclusion criteria** **# significant baseline difference**	**N (% retention)** **Age** **Gender**	**Self-Care Program and equipment supplied (if any)** **Comparator groups (if any)**	**Monitoring** **Measurement Interval**	**Outcome** **(Outcome measure)** ***significant benefits reported**
Jullien et al, (2011) Africa People enrolled in the National 'Washing' Project'	Prospective Uncontrolled Mean follow up 4.5 months (range 1-5)	Limb not specified ≤ 1 clinic visits in 4.5 months # age of retained vs lost subjects	n = 1578 (69%) Mean years 46.6 (Range 10 - 98) Female = 74.50%	Washing Program: Wash and dry limb up to 4 times per day, clip nails, use footwear Basin, cup, soap & medication and leaflet to identify ADLA provided	Train 1 health care worker per 15 patients Attend clinic 1-5 times Measured at: Baseline and clinic visits (range 2 – 6 total visits)	ADLA frequency* (Recall previous month) Frequency of consultations (Clinic records) ADLA by frequency of consultation*
Addiss et al. (2010) Haiti Hospital clinic	Prospective, 2 groups Intervention: Minimum 6 months Follow up: Mean 22.1 months (range 6.3 – 41.2) Group 2 n = 48	Leg (s) ≥ 5 clinic visits Stage 0-4 (Dreyer 2002) # Mean ADLA	n = 175 (100%) Mean 37.3 years (Range 10 – 85) Female = 82.9%	Hygiene program: Education, wash legs, ROM exercises, elevation, oral and topical medication as required Equipment, antibiotic cream, instruction booklet provided Group 1 = Therapist treatment Group 2 = Self-care	Visit clinic: Every 4-6 weeks or during ADLA Ad hoc home visits Measured at: Baseline and every clinic visit	ADLA frequency* (Recall previous 12 months) (Observed) Limb volume* (Water displacement) Compliance (Questionnaire)
Wijesinghe et al (2007) Sri Lanka Two filariasis clinics	Prospective Uncontrolled Intervention: 12 months	Leg(s) Arm(s) ≥ 6 weeks swelling ≥ 5 years old	n = 413 (39.5%) Arm(s) = 1.7% Leg(s) = 98.3% ≤ 35 years = 6% 36 - 65 years = 80% > 65 years = 14% Female = 81.6%	Community Based Home Care: Individual training of patient or carer, twice daily washing, keep dry, elevation, ankle exercises, use footwear, prevent trauma, treat entry lesions, oral and topical medication for ADLA Patient booklet provided	Visit clinic: Monthly if experiencing symptoms Measured at: Baseline and 12 months	Change in lymphedema grade* (Examination, WHO 1992) Number of entry lesions* (Examination) ADLA frequency*, duration, and ADLA management practices* (Recall previous 12 months) Perceived improvement (Interview)
Wilson et al (2004) Haiti Hospital clinic	Prospective Within subject control Intervention: Mean days 365	Leg(s) No ADLA previous 2 weeks Stage 1 – 3 (WHO 1992) Residing within	n = 91 (29.7%) Median 39 years (Range 16 - 75) Female = 80%	Lymphedema Self-care; Daily washing, ROM exercises, elevation, prevent entry lesions Antimicrobial medication and symptomatic treatment during ADLA Soap, towels, wash basin and cream provided	Attend clinic or home visit: Every 4 – 6 weeks or during ADLA Biopsy follow up between 317 - 656 days	Lymphedema stage (Examination, WHO 1992) ADLA frequency* (Recall previous 12 months) (Observed) Histologic Changes* (Skin-punch biopsy, 4 mm)

Study		Population		Intervention		Outcomes
Author (Year) **Country** **Setting**	**Design** **Duration and Follow Up** **Groups in Review**	**Affected limb (s)** **Inclusion criteria** **# significant baseline difference**	**N (% retention)** **Age** **Gender**	**Self-Care Program and equipment supplied (if any)** **Comparator groups (if any)**	**Monitoring** **Measurement Interval**	**Outcome** **(Outcome measure)** ***significant benefits reported**
		10km of the clinic	Affected vs unaffected histological analysis n = 26			
Bernhard et al (2003) Tanzania Lymphedema clinic	Prospective 2 groups Group 2, n=25 Intervention: 9 months	Leg(s) ≥ 6 months swelling ≥ 18 years old Able to reach own toes for bandaging	n = 46 (79.7%) Mean years (SD) Female = 47(14.3) Male = 42(16.6) Female = 63%	Self-treatment: Instruction, daily wash and dry, SLD, elevate day and night, exercise, use footwear, avoid injury, medical treatment for ADLA Bandages, compression garments and illustrated leaflet provided Compression bandaging for 3 weeks then ongoing compression garment: Group 1 = Therapist treated Group 2 = Self-treated	Attend clinic: Weeks 2 and 3 3, 6 and 9 months Measured at: Baseline, 3 weeks, 3, 6 and 12 months	Limb Volume* (circumference measures at 4cm intervals) Entry wounds and fungal infections (Observed)
McPherson T (2003) Guyana One municipality	Prospective Uncontrolled Intervention: 12 months	Limb(s) not stated FR-LE patients within the community receiving no care	n=14, FU = 79% Mean 47 years (Range 21 - 65) Female = 81.8%	Train a specialist nurse within a hospital clinic Morbidity reduction: Hygiene, skin-care, elevation, simple exercises, oral antibiotics for ADLA, antibacterial and antifungal cream as needed Patient education leaflet provided	Nurse available for advice and support Measured at: Baseline and 12 months	Quality of Life* (Dermatology Quality of Life Index) ADLA (Recall 6 months) Stage (Dreyer et al 2002)
Suma et al (2002) India Hospital Clinic	Retrospective Uncontrolled Intervention: 12 months	Leg (s) Arm (s) Participants of a previous RCT (Shenoy et al 1999)	n = 127 Median 45 years (Range 18 - 67) Female = 56.7%	Foot-care: Nightly cleaning, keep dry, clip nails, apply ointment between toes and sides of feet, antibiotic cream as needed, use footwear	Nil monitoring Baseline data from previous RCT Measured at 12 months	ADLA frequency (Recall 12 months) Entry lesions (Clinical assessment)

#= Statistically significant differences between groups at baseline
*= Statistically significant improvement reported for this outcome

SD = standard deviation
NGO = Non Government Organization
WHO = World Health Organization

WHODASII = WHO Disability Assessment Scale II
ADLA = Acute dermato lymphangio adenitis

ROM = range of motion
ADLA = Acute dermato lymphangio adenitis

Table S2.3: Description of Randomized Controlled Trails (RCT) on CR-LE

Study		Population		Intervention		Outcomes
Author (Year) **Country** **Setting**	**Design** **Duration and Follow Up** **Groups in Review**	**Affected limb (s)** **Inclusion criteria** **# significant baseline difference**	**N (% retention)** **Age** **Gender**	**Self-Care Program** **Equipment supplied (if any)** **Comparator groups (if any)**	**Monitoring** **Measurement Interval**	**Outcome** **(Outcome measure)** ***significant benefits reported**
Letellier (2014) Canada University health centre and private lymphedema clinic	RCT, 2 groups Intervention: 12 weeks Group 1, n =12	Unilateral Arm Stratified by relative limb volume of < 25% / ≥ 25% # Onset after surgery, duration of lymphedema	n = 25 (72%) Mean years (SD) Group 1, 53.4 (9.35) Group 2, 56.4 (9.76) Female = 100%	Home exercise; SLD, corrective and strengthening exercises, compression sleeve DVD of home exercises provided Group 1 = Home exercise Group 2 = Home exercise and weekly aquatic exercises classes	Log book Measured at: Baseline and 12 weeks	Relative limb volume (Water Displacement) (Circumference at 4cm intervals) Grip strength* (Dynamometer) Perceived function* (DASH) Pain (Short-form Magill questionnaire) Quality of Life* (FACT –B)
Jeffs and Wiseman (2013) UK Hospital clinic	RCT, 2 groups Intervention: 6 months Group 1, n = 11 Group 2, n = 12	Unilateral Arm Relative limb volume ≥ 10%	n =23 (100%) Median years (LQ, UQ) Group 1, 66 (51, 63) Group 2, 64.5 (56, 73.5) Female = 100%	Standard care: Daily including skin-care, exercise, hand pumping, compression sleeve Home based exercise: Resistance exercise, deep breathing, compression New compression garment and illustrated instruction sheet provided Group 1= Standard care + Home based exercise Group 2 = Standard care	Measured at: Baseline, 4, 12 and 26 weeks	Relative limb volume* (Perometry) ROM (Goniometer) Quality of Life (LYMQoL) Perception of function (Quick DASH-9) Adherence (Self-report)

Study		Population		Intervention		Outcomes
Author (Year) **Country** **Setting**	**Design** **Duration and Follow Up** **Groups in Review**	**Affected limb (s)** **Inclusion criteria** **# significant baseline difference**	**N (% retention)** **Age** **Gender**	**Self-Care Program** **Equipment supplied (if any)** **Comparator groups (if any)**	**Monitoring** **Measurement Interval**	**Outcome** **(Outcome measure)** ***significant benefits reported**
Barclay et al (2006) UK Hospital clinic	RCT, 2 groups Intervention: 6 months Group 1, n = 40 Group 2, n = 41	Leg(s) Arm(s) Stratified by age and affected limb ≥ 18 years Able to perform self-massage	n = 81 (92.6%) unilateral arm = 61 bilateral leg = 20 25 - 80 years Female = 95%	Standard care: Daily SLD, exercise, skin care, compression garment Daily self-massage: Group 1= Plain massage cream Group 2 = Aromatherapy massage cream	Measured at: Baseline, 1, 2, 3 and 6 months	Limb volume* (Circumference at 4cm intervals) Symptoms and wellbeing* (MYMOP2)
Andersen et al (2000) Denmark Hospital clinic	Randomized crossover trial 2 groups Intervention: 3 months Follow up: 12 months Group 2, n = 22	Unilateral arm Relative limb volume ≥ 200ml # Endochrine therapy	n = 42 (92.86%) Median 53 years (Range 25 - 77) Female = 100%	Standard therapy: instruction, SLD, exercise, skin care and safety precautions Custom made sleeve and glove provided Group 1 = Therapist massage Group 2 = Self-massage	Measured at: Baseline, 1, 3, 6, 9 and 12 months Results at 6, 9 and 12 months not included in review	Relative Limb Volume* (Circumference at 5cm intervals) Self-reported Symptoms (Interview) Compliance (interview)

#= Statistically significant differences between groups at baseline
*= Statistically significant improvement reported for this outcome
SD = standard deviation
LQ = Lower quartile
UQ = Upper quartile
LYMQol = Quality of Life Measure for Limb Lymphoedema (Keeley et al 2010)

DASH = Disabilities of the arm, shoulder and hand, www.dash.iwh.on.ca
Quick DASH 9 = Disabilities of the arm, shoulder and hand, www.dash.iwh.on.ca
SLD = Self lymphatic drainage (massage)
FACT B = Functional assessment of cancer therapy – breast www.facit.org
MMYOP2 = Make yourself medical outcome profile version 2 www.measuringimpact.org

Table S2.4: Description of Cohort Studies on CR-LE

Study		Population		Intervention		Outcomes
Author (Year) **Country** **Setting**	**Design** **Duration and Follow Up** **Groups in Review**	**Affected limb (s)** **Inclusion criteria** **# significant baseline difference**	**N (% retention)** **Age** **Gender**	**Self-Care Program** **Equipment supplied (if any)** **Comparator groups (if any)**	**Monitoring** **Measurement Interval**	**Outcome** **(Outcome measure)** ***significant benefits reported**
Johansson et al (2014) Sweden Hospital clinic	Prospective Uncontrolled New sleeve: 2 weeks Control period: 2 weeks Intervention: 12 weeks	Arm unilateral ≥ 6 months swelling Relative limb volume ≥ 200ml or ≥ 2cm > 70 years	n = 26 (88.5%) Mean 58 years (SD 8.0) Female = 100%	Progressive resistance exercise for 4 weeks Home weight lifting exercise for 8 weeks New compression garment provided	Fortnightly review of log book Measured at 2, 4, 6 and 14 weeks	Relative limb volume* (Water Displacement) Extracellular fluid ratio (Bio impedance spectroscopy) Isometric Muscle Strength* (Strain gauge) Grip strength (Hand dynamometer) Perceived disability (DASH)
Jonsson and Johansson (2014) Sweden Hospital clinic	Prospective Uncontrolled New sleeve: 2 weeks Intervention: 8 weeks	Arm unilateral >75 years Relative limb volume ≥ 10% Palpable tissue changes or subjective symptoms	n = 35 (65.7%) Mean 60.4 years (SD 8.3) Female =100%	Pole walking with light arm exercise 3-5 times per week New compression garment	Log book Fortnightly clinic visit Measured at baseline, 2, 4, 6, 8 and 10 weeks	Relative limb volume* (Water Displacement) Cardiovascular fitness* (Bicycle ergonometer) Perceive disability (DASH) Self-reported symptoms* (Likert scale) General wellbeing (Questionnaire)
Douglass et al. (2012) Australia Hospital clinic	Retrospective 2 groups Follow up: 6 months Group 1, n = 9 Group 2, n = 9	Unilateral arm Participants of a previous RCT on a 4 week yoga program	n = 18 Mean years (SD) Group 1, 65.0 (12.4) Group 2, 60.4 (11.1) Female = 100%	Self-management: Education, SLD, exercise, skin care, compression sleeve Home yoga program; Daily exercise, breathing exercise, meditation Illustrated instruction sheet and CD-Rom of guided relaxation provided Group 1 = Continued home yoga Group 2 = Discontinued home yoga	Baseline (data from RCT) Measured at 6 months	Relative limb volume (Bio impedance spectroscopy) (Perometry) Tissue compressibility (Tonometry) Self-reported symptoms (Likert scale) Quality of Life (Visual Analogue Scale)

Study		Population		Intervention		Outcomes
Author (Year) **Country** **Setting**	**Design** **Duration and Follow Up** **Groups in Review**	**Affected limb (s)** **Inclusion criteria** **# significant baseline difference**	**N (% retention)** **Age** **Gender**	**Self-Care Program** **Equipment supplied (if any)** **Comparator groups (if any)**	**Monitoring** **Measurement Interval**	**Outcome** **(Outcome measure)** ***significant benefits reported**
Gautam et al (2011) India Hospital clinic	Prospective Uncontrolled Intervention: 8 weeks	Unilateral Arm Relative limb volume ≥ 200ml or ≥ 2cm Stage I and II (Criteria not given)	n = 38 (84.2%) Mean 46.6 years (SD 6.98) Female = 100%	Self-care; Education, SLD, skin-care, elevation, avoid injury, compression sleeve Home based exercise program: Progressive resistance exercise and deep breathing Instruction sheet provided	Weekly phone call Clinic or home visit at 4 weeks Measured at: Baseline, 4 and 8 weeks	Circumference* (Difference between affected and unaffected arm at 4 places) Relative limb volume* (Water Displacement) Quality of Life* (Short Form Health Survey 36)
Koul et al (2007) Canada Lymphedema clinic	Retrospective 3 groups Group 3, n= 18	Unilateral arm ≥ 1 year follow up # severity of lymphedema	n = 138 Mean years 54.3 (range 29 - 82) Female = 100%	Training in SLD, skin-care and remedial exercise Custom made compression sleeve provided Treatment allocation at discretion of therapists: Group 1 = Therapist treatment including bandaging, deep breathing Group 2 = Therapist treatment without bandaging Group 3 = Self-care with SLD and deep breathing	Telephone interview at 3 months Measured at: 6 and 12 months	Relative Limb Volume* (Circumference at 4cm intervals)
Moseley et al (2005) Australia Hospital clinic	Prospective Retrospective 2 groups Group 1, n= 40 Group 2, n = 28 (Control cases from a previous study)	Unilateral Arm ≥ 6 months swelling Relative limb volume ≥ 200ml	n = 68 (76.5%) Mean years (SD / range) Group 1, 60.1 (1.6 / 42.-76) Group 2, 65 (2.0 / 42-87) Female = 100%	Deep breathing exercise: Gentle arm extension with each breath, 5 breaths and 1 minute rest, repeated for 5 cycles Group 1 = Deep breathing exercise Group 2 = Retrospective controls	Log book Measured at: Baseline, every 10 minutes first hour, 24 hours, 1 week, 1 month	Relative limb volume* (Perometry) Truncal fluid (Bio impedance spectroscopy) Tissue compressibility (Tonometry) Self-reported symptoms* (Likert scale)

#= Statistically significant differences between groups at baseline
*= Statistically significant improvement reported for this outcome
SD = standard deviation
DASH = Disabilities of the arm, shoulder and hand, www.dash.iwh.on.ca
SLD = Self lymphatic drainage (massage)

S3 Effect of Interventions on Filariasis Related Lymphedema (FR-LE)

Effect of Basic Self-Care on ADLA

Table S3.1: Frequency of ADLA episodes after 6 - 24 months of basic self-care

Study ID	Group(s)	Baseline		Treatment Period		% change
		Mean	N	Mean (range)	N	
Mues et al 2014	Self-care cohort	0.35 *	370	6 months 0.14	324	Reduced 60%
				12 months 0.23	321	Reduced 35% from Baseline
				24 months 0.23	316	Reduced 35% from Baseline
Addiss et al 2010	Self-care cohort	1.31 (range, 0-6)	48	12 months 0.22	48	Reduced 83% p=0.0001
Akogun & Badaki 2011	Community care group	3.8	299 (limbs)	0	91 (limbs)	Reduced 100%
	Patient care group	2.6	137 (limbs)	0	7 (limbs)	Reduced 100%
	Health facility group	3.4	237 (limbs)	2.5	2 (limbs)	Reduced 26%
Wilson et al 2004	Participants who consented to a second skin biopsy	1.7 (range, 0-8)	91	0.5 (range, 0-3)	26	Reduced 71%
Suma et al 2002#	Unsupervised year	1.7 (range, 1-12)	127	2.8 (range, 1-42)	127	Increased 65%
	Baseline from 1999 drug trial	4.7 (SE 0.7)	150	2.8 (range, 1-42)	127	Reduced 40%

** Rate per person month, # Baseline data is from 2nd year of follow up in Shenoy et al 1999, SE = standard error*

Table S3.2: Reduction in frequency of ADLA episodes by Stage of FR-LE after 6 - 24 months of basic self-care.

Study	LE Stage	Baseline	N	6 months	N	% Change	12 months	N	% Change	24 months	N	% Change
Mues et al 2014* Rate per person month	1 – 3	0.29	317	0.1	324	- 65.5%	0.19	321	- 34.5%	0.2	316	- 31.0%
	4 - 6	0.78	53	0.22		- 71.8%	0.42		- 46.2%	0.46		- 41.0%
Das et al 2013 Mean episodes	I	2.4	10				0.8	10	- 66.7%			
	II	3.4	50				1.2	47	- 64.7%			
	III & IV	4.8	36				1.8	36	- 62.5%			
Wijesinghe et al 2007 Mean (SD) episodes	I	0.36 (0.74)	14				0.21 (0.43)	26	- 41.7%			
	II	0.99 (1.68)	86				0.19 (0.54)	75	- 80.8% [1]			
	III & IV	2.78 (7.92)	63				0.52 (1.56)	62	- 81.3% [2]			
Suma et al 2002# Mean episodes	I	0.4	127				1.4	127	+ 40.0%			
	II	0.8					1.6		+ 100%			
	III	1.6					2.8		+ 75.0%			
	IV	3.0					4.4		+ 46.7%			

[1] *p<0.001*

[2] p=0.022

 # Baseline data from 2n`d year of observation Shenoy et al (1999)
SD = standard deviation

Table S3.3: Duration of ADLA episodes after 12 months of self-care for FR-LE

Study ID	Previous 12 months		Treatment Period		% Reduction
	Mean days	N	Mean days	N	
Das et al 2013	4	93	2.5	93	37.5%
Wijesinghe et al 2007	5.8 (SD 3.97, range 1-30, mode 7)	163	5.7 (SD 3.4, range 2-14, mode 3)	163	24.0% RR 0.14 (95%CI 0.94, 0.56)

SD = standard deviation

Table S3.4: Duration of ADLA episodes in the previous 6 months by percentage of participants

Study ID	Duration in days	Baseline		After 12 months		% Change
		% participants	N	% participants	N	
Akogun & Badaki 2011	0	6%	299 (limbs)	83.5%	91 (limbs)	Increased 92.8%
	1-3	39.8%		9.9%		Reduced 75%
	4-6	30.8%		6.6%		Reduced 78.6%
	≥7	23.4%		0%		Reduced 100%

Table S3.5 Days of work lost in the preceding 30 days after enrolment in a CBHC program

Study	Stage	Time point			% Reduction	
		Baseline	6 months	24 months		
Budge et al 2013	All stages	6.4 (95% CI: 5.6, 7.2)	2.9 (95% CI: 2.4, 3.4)	3.9 (95% CI: 3.2, 4.6)*	39%	*sig lower than baseline
	Advanced (4-7)	10.4		5.9	44%	p=0.0083
	Moderate (3)	6.2		4.5	28%	p=0.0439

Effect of basic self-care on Perceived Disability and Quality of Life in FR-LE

Table S3.5: Change in perceived disability and quality of life after 12 – 24 months

Study ID	Stage*	Before	N	After	N	Duration	Improvement
McPherson T 2003[1] Mean score (range)	All stages	10.2 (2-18)	14	4.1 (0-11)	11	12 months	6.8 (0-15) p≤0.0001
Budge et al 2013[2] Composite score	1 – 2	60.1	184	57.4	188	24 months	4.5% p=0.0697
	3	69.0	133	62.0	82	24 months	10.1% p=0.0011
	4 – 7	80.2	53	69.9	46	24 months	12.8% p=0.0044

** Stages per Dreyer et al 2002*
1=Dermatology Quality of Life Index (DQLI)
2=WHO Disability Assessment Schedule II (WHO DAS II) (WHO, 2001)

Effect of self-care with medicated/plain cream or soap for FR-LE

Table S3.6: Reduction in mean ADLA - RCTs on cream or soap

Study ID	Group	Previous year		Treatment year			Follow up year		
		Mean	N	Mean	N	% Reduction*	Mean	N	% Reduction*
Joseph et al 2004	Medicated cream	2.43	30	0.56	27	76.95% #	0.85	27	65.02% #
	Plain cream	2.63	30	0.59	27	77.57% #	0.44	27	83.27% #
Shenoy et al 1999	Medicated cream	4.2	30	1.1	29	73.81% p<0.001	1.7	29	59.52% p<0.001
	Plain cream	4.7	30	1.7	28	63.83% p<0.001	1.0	28	78.72% p<0.001
Shennoy et al 1998	Medicated cream	3.63	40	1.25	39	65.6% p<0.001	0.98	30	73.0% p<0.001
Kerketta et al 2005	Medicated cream	3.2	100	1.2	84	62.5% #	n/a	n/a	n/a
Addiss et al 2011	Medicated & plain soap	1.1	200	0.4	197	63.3% #	n/a	n/a	n/a

** Reduction from baseline*
 # significance not given
n/a = no follow up in this study

Table 15. Reduction in leg volume (litres)*

Study ID	Self-bandaging period		Compression garment period					
	After 3 weeks		After 3 months		After 6 months		9 months	
	Litres (range)	**N** (legs)	**Litres (range)**	**N** (legs)	**Litres (range)**	**N** (legs)	**Litres (range)**	**N** (legs)
Bernhard et al 2003	0.85 (-0.12 to 5.75) p=0.049	33	0.61 (0.12 to 7.55) p=0.003	31	0.48 (-0.06 to 7.55) p=0.001	32	0.32 (-0.03 to 5.77) p=0.016	25

** Limb volume calculated from circumference measures at 4 cm intervals*

S4 Tables: Effect of Self-Care on CR-LE

Table S4.1: Effect of home-based exercise on arm volume

Study ID	Exercise	Duration	Intervention Group		Controls		Measure
			Change	N	Change	N	
Gautum et al 2011	Isotonic exercise, deep breathing	8 weeks	Reduced mean 122.83mls (+/- 30.37) $p < 0.0001$	38	Within subject control[1]	n/a	Water displacement
Jeffs & Wiseman 2013	Gravity resisted exercise	12 weeks	Reduced 8.08% $p = 0.05$	11	Reduced 2.83% $p = 0.041$	12	Perometry
		26 weeks	Reduced 11.69% (95% CI -26.57, -5.12) $p = 0.013$	11	Reduced 9.2% (95% CI -17.71, 1.1)	12	
Moseley et al 2005	Deep breathing, gentle arm exercise	10 minutes	Reduced mean 52mls (5.8%) $p = 0.004$	24	Within subject control[1]		Bio-impedance spectroscopy, Perometry
		1 month	Reduced 101mls (9%)	24	Increased 7ml #	28	
Douglass et al 2012	Home yoga program	6 months	Reduced 14.3%	9	Increased 25.4%	9	Bio-impedance spectroscopy
		6 months	Reduced 9.8%	9	Increased 16.7%	9	Perometry

 # Controls from a previous study
1 = Uncontrolled cohort study

Appendix F2: Reliability study

Douglass, J., Graves, P., Gordon, S., *Intrarater Reliability of Tonometry and Bioimpedance Spectroscopy to Measure Tissue Compressibility and Extracellular Fluid in the Legs of Healthy Young People in Australia and Myanmar.* Lymphatic Research and Biology, 2017. **15**(1): p. 57-63.

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Intrarater Reliability of Tonometry and Bioimpedance Spectroscopy to Measure Tissue Compressibility and Extracellular Fluid in the Legs of Healthy Young People in Australia and Myanmar

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Abstract

Background: Measurements of tissue compressibility and extracellular fluid (ECF) are used to monitor progression of lymphedema, a chronic swelling of the subcutaneous tissue. Later stages of lymphedema are characterized by fibrotic induration in the subcutis and hyperkeratosis of the skin. Several devices are available to measure these changes, but previous reliability and validity studies have been conducted primarily on adult women with unilateral arm lymphedema using contralateral limbs as controls. To date, no studies have included either adolescents or measurement of leg tissue.

Methods and Results: An intrarater reliability study was conducted to compare three devices measuring skin and subcutaneous tissue compressibility; a mechanical Tonometer, a digital Indurometer, and a SkinFibroMeter. ECF loads were measured using bioimpedance spectroscopy (BIS). Two populations of tropical-dwelling young people were included; Australian residents in North Queensland aged 8–21 years ($n = 34$) and people aged 10–21 years residing in Central Myanmar ($n = 38$). Neither cohort had any clinical sign of lymphedema or other leg abnormality. The mechanical Tonometer and the digital Indurometer had excellent intraclass correlation coefficient (ICC) scores between 0.792 (95% CI 0.055–0.901) and 0.964 (95% CI 0.945–0.984) and the SkinFibroMeter had good to excellent reliability with ICC scores of between 0.565 (95% CI 0.384–0.747) and 0.877 (95% CI 0.815–0.840). BIS exhibited the highest reliability with ICC scores approaching 1.0.

Conclusions: These results support the reliable use of tonometry and BIS to assess tissue compressibility and ECF loads in the legs of adolescent populations in developed and developing tropical countries.

Introduction

Lymphedema is the external manifestation of an overwhelmed lymphatic system. Extracellular fluid (ECF) and circulating proteins are not adequately removed from the tissue spaces, and over time, the excess protein-rich fluid is replaced by an accumulation of fibrosis and fatty tissue, particularly in the subcutaneous compartment. As lymphedema progresses, the affected body part becomes enlarged, the skin thickens (hyperkeratosis), and eventually takes on the characteristic appearance of elephantiasis.[1]

Globally, the greatest cause of lymphedema is lymphatic filariasis (LF), a nematode worm transmitted by mosquitoes in 73 tropical countries. It is estimated that 16 million of the world's poorest people have LF-related lymphedema (LFRL).[2] Lymphedema also forms after treatment for some cancers and breast cancer-related lymphedema (BCRL) affects between 17% and 28% of breast cancer survivors.[3] Whether LF or cancer related, progression of lymphedema can be slow and persistent or may advance rapidly as a consequence of repeated bacterial or fungal infections. During the onset and progression of lymphedema, tissue compressibility and ECF loads change along a predictive course.[4] Devices commonly used to measure changes related to BCRL include tissue tonometry to quantify compressibility of affected tissue and bioimpedance spectroscopy (BIS) to quantify ECF fluctuations.[5] Assessment of LFRL more frequently depends on clinical staging by presentation and history with historically limited use of tools to quantify changes.[6]

Use of tissue tonometry to provide an objective measure of compressibility in the skin and subcutis was first reported in

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1976.[7] It provides an objective measure of the changes in tissue composition, which occur over the course of lymphedema. In the early fluid-rich stage, the tissue is soft and easily compressible, but as lymphedema progresses, the skin and underlying tissues become progressively thicker and harder and compressibility will decrease. Several versions of the mechanical Tonometer and recently developed digital devices (the digital Indurometer and the SkinFibroMeter) are now available. Increased ECF loads in lymphedema have been measured using BIS since 1996[8] and several devices are in regular use in both clinical and research settings.[9]

Previous studies have shown tonometry and BIS to be reliable in measuring changes associated with lymphedema, but most were in middle-aged women with unilateral BCRL of the arm using the contralateral arm as a control.[10–14] More recently, attention has been directed toward detecting subclinical changes, which occur before lymphedema can be observed clinically, and BIS has been shown to be a reliable tool for this purpose in women at risk of BCRL.[15] To date, only BIS has been used on infants.[16] Few lymphedema studies have included people with leg lymphedema[17] and although human growth and aging may influence measures, no reliability studies have been conducted on young people.[18]

Confident and defensible use of these tools to diagnose and monitor lymphedema is dependent on establishing whether they are reliable for use with populations of need. While lymphedema is essentially the same disease regardless of the cause, LFRL affects a much larger and demographically different population than women in developed countries with BCRL. Most commonly leading to lymphedema of the legs, LFRL can affect both men and women at any age, young, middle aged, and older.[6]

The aim of this study was to identify the intrarater reliability of three different tissue tonometers and one BIS device to assess the lower limbs of young people. Populations from two different ethnic backgrounds were included to inform the methodology of a forthcoming study to detect subclinical lymphatic change in young people living in a filariasis endemic region in Myanmar.

Materials and Methods

The Guidelines for Reporting Reliability and Agreement Studies were used to inform the study design and reporting.[19] A convenience sample of young people from Townsville, Australia, and Amarapura Township, Mandalay Region, Myanmar, were invited to participate in the study. In Australia, people aged 8–21 years were recruited through institutional emails and local notice boards. In Myanmar, young people aged 10–21 years were recruited with the assistance of local health workers and residential block leaders. Participants were excluded if they had any clinical sign of lymphedema or any injuries to their legs. The Myanmar cohort was screened for LF infection using immunochromatographic test cards, and young people who tested positive were excluded from the reliability analysis. Ethical approval for the study was provided by the James Cook University Human Research Ethics Committee (approval number H5497) and by the Myanmar Ministry of Health and in accordance with the Helsinki Declaration as revised in 2008. Consent to participate in the study was given by young people aged 18–21 years and by a parent or guardian of minors aged 8–17 years. The parent or guardian also accompanied the child during the measurement session.

Height was recorded in centimeters and weight was recorded in kilograms. All tissue tonometry measures were taken at the midpoint of the anterior thigh, posterior thigh, and calf of each lower limb. A tape measure was used to obtain three length measurements: the calf between the base of the heel and the crease behind the knee, the posterior thigh between the crease behind the knee and the gluteal fold, and the anterior thigh between the superior border of the patella and the crease of the groin. The midpoint of each segment was then calculated by halving the full-length measures and a washable skin marker was used to mark each midpoint on the leg. All tonometry measures were taken at the marked points by a qualified lymphedema practitioner experienced in the use of tonometry (JD). In Myanmar, local health assistants collected informed consent, recorded height, weight, infection status, and interview responses. Verbal assent to take the measures was reiterated before any leg measurements were taken. Limb dominance, which has been reported to alter BIS,[11] was determined by asking the following question: Which foot do you use to kick a ball? Blinding was not possible as all measures were taken by a single operator, but a sequential order of measures was followed in both groups to minimize the risk of bias.

The mechanical Tonometer (Flinders Biomedical Engineering, Australia) is a handheld device with a 275 g mass above a 1 cm diameter indenter, which extends through an opening in a 6 cm diameter reference plate and applies a differential pressure of 263 g/cm^2. With the reference plate resting lightly on the skin, the weighted indenter presses into the skin and underlying tissue and the degree of tissue resistance is shown on an analog displacement gauge in 0.1-mm increments. Higher values indicate softer tissue as the indenter is able to press deeper into the tissue.

The Indurometer (Flinders Biomedical Engineering, Australia) is a digital version of the mechanical Tonometer utilizing a constant force spring to deliver a 200 gm pressure. The reference plate rests evenly on the surface of the skin and the device is pressed into the tissue. A beep is emitted once the 200 gm force has been achieved. The result is displayed digitally in 0.01-mm increments, which can be read from the digital display after the measure is completed; higher values indicate softer tissue.

The SkinFibroMeter (Delfin Technologies, Finland) has a smaller reference plate (diameter 2 cm) and a 1-mm-long indenter and records the resistance to 50 gm of pressure. The device is applied gently five times in the same location and the digital readout displays the average measure of resistance in Newtons (N), a lower value indicating less resistance or softer tissue.

Bioimpedance was measured using the SFB7 (Impedimed, Queensland), a portable, battery-operated multifrequency analyzer, which delivers 256 frequencies over the range 3–1000 KHz. Low frequencies pass through the ECF, while higher frequencies pass through both the ECF and the intracellular fluid (ICF). An increase in the Ri:Re (intracellular impedance: extracellular impedance) ratio indicates an increase in ECF load.[20]

Although persistent indentation should not occur in healthy skin, the tonometry instruments were used in the following order to avoid any residual pitting from the previous device: (1) The SkinFibroMeter, which applies only 50 gms of pressure; (2) the Indurometer, which requires 200 gms of pressure and is

applied only briefly; and (3) the mechanical Tonometer, which also applies 200 gms, but must remain on the skin while the operator reads two analog scales. All measures were taken three times with each device in the order right calf, left calf, right posterior thigh, left posterior thigh, right anterior thigh, and left anterior thigh. The SkinFibroMeter and Indurometer were used with both groups and the mechanical Tonometer was used with the Myanmar group only.

The BIS measures were collected last. Self-adhesive electrodes were placed on the skin at the ankles and the hands according to the manufacturer's instructions for whole leg analysis. Both legs were measured three times beginning with the right leg and the Ri (ICF resistance) and Re (ECF resistance) values for each measure were recorded.

All analyses were performed using STATA 12 (StataCorp.). A *t*-test was used to compare group characteristics such as height and weight. As the data were continuous, an intraclass correlation coefficient (ICC) was used to determine the intrarater reliability or test–retest reliability. This test indicates the reliability of each measurement device to differentiate participants under repeated similar assessment conditions.[19] A one-way repeated measures analysis of variance was used to obtain within-subject variance and hence overall average ICC for the four devices at each measurement location. Higher ICC represents better intrarater reliability, hence an average ICC score of 0 represents no correlation and 1 equals absolute agreement. An ICC of less than 0.4 is considered to indicate poor agreement between measures, between 0.4 and 0.75 is fair to good agreement, and greater than 0.75 is excellent.[20] A sample size estimation of 62 subjects was based on an expected correlation coefficient of 0.4 with a 95% confidence interval using an online calculator (http://biomath.info/power/corr.htm).

Each device uses a different scale, which prevents direct comparisons of the overall means and variance of the values recorded; so, to determine the agreement between devices at each measuring location, a coefficient of variation (CoV) [19] was calculated using the following formula:

$$\text{CoV} = \frac{\text{standard deviation (SD)}}{\text{Overall mean for the device}} \times 100$$

The CoV is reported as a percentage, a lower score indicating less variation (more agreement) between devices.

Results

Thirty-four young Australians and 38 young Myanmar people were included in the study. Despite including younger children in the Australian group, there was no significant difference in the mean age, gender, or leg dominance between the two groups. The majority of participants were female (60.4%), the mean age (range) was 15 years (8–21), and 95.6% were right leg dominant. The Australian group was significantly taller and heavier than the Myanmar group, but there was no statistically significant difference in BMI (Table 1).

Indurometry of the anterior thigh and BIS measurements were not recorded for one Australian participant. Poor electrode contact affected a small number of BIS measures, and if any single measure was affected, the remaining two measures in that measurement set were also discarded. This meant that there were bilateral BIS measures available for 32 Australian and 36 Myanmar participants, with dominant leg values available for all Myanmar people and nondominant leg values available for 33 Australians.

The midpoint of the anterior thigh recorded the softest measures by all three devices (highest mean values by mechanical Tonometer and Indurometer, lowest mean values by SkinFibroMeter). The hardest tissue was consistently recorded at the midpoint of the calf, and the mean values for the posterior thigh fell between these two ranges for all devices. The BIS ratio of resistance, ICF:ECF, was slightly higher in the nondominant leg in both populations, indicating that this leg had slightly more ECF than the dominant leg, but this was not significant. Mean values and SDs for each device at each measuring point are provided as supplementary material (Supplementary Table S1; Supplementary Data is available in the online article at www.liebertpub.com/lrb).

Of the tissue tonometers, the mechanical version (used only in the Myanmar group) had the best ICC (0.893–0.964) and CoV (10.8%–20.4%) scores. Excellent ICC scores were also recorded for the Indurometer (0.792–0.956) with low (good) CoV of 14.8%–32.2%. The SkinFibroMeter had good to excellent ICC scores (0.565–0.877); however, this device scored the highest (poorest) CoV (18.1%–43.1%). The ICC scores for BIS approached 1.0 for all measures (ICC = 0.912 to >0.999) and the CoV was very low (2.3%–20.8%). The ICC, estimated reliability, and CoV scores for each device and group are reported in Table 2.

Both the mechanical Tonometer and the Indurometer had better ICC scores when used to measure the posterior thighs and calves than when used to measure the anterior thighs in both populations. ICC scores for the SkinFibroMeter were better in the Myanmar group than the Australian group, but in contrast to the other devices, the best ICC scores were on the anterior thigh. The relationship between anatomical location of the measuring points and the ICC score is demonstrated in the radar graph in Figure 1. The data closer to the outside

Table 1. Age, Height, Weight, and BMI of Participants in Myanmar and Australia

	Myanmar n = *38*			*Australia* n = *34*			t-*test*
	Mean ± SD	*Range*	*95% CI*	*Mean*	*Range*	*95% CI*	*p*
Age in years	15.8 ± 3.1	10–21	14.8–16.8	14.7 ± 3.9	8–21	13.4–16.0	0.172
Height in cm[a]	154.2 ± 9.7	121–168	151.1–157.3	160.2 ± 14.3	130–187.5	155.3–165.1	0.039
Weight in kg[a]	45.2 ± 9.4	21.3–71.2	42.1–48.2	52.8 ± 16.1	23.2–86.1	47.3–58.3	0.016
BMI	18.8 ± 2.7	13.7–27.5	17.9–19.7	20.0 ± 3.3	12.4–28.1	18.9–21.2	0.088

[a]significant between-group differences.
SD, standard deviation.

Table 2. ICC and CoV for Each Device at All Measurement Points by Population Group

	Myanmar n = *38*			*Australia* n = *34*		
	ICC (95% CI)	*CoV %*	n	*ICC (95% CI)*	*CoV %*	n
SkinFibroMeter						
Anterior thigh dominant leg	0.640 (0.489–0.792)	24.2	38	0.659 (0.504–0.814)	18.1	34
Anterior thigh nondominant leg	0.877 (0.815–0.940)	26.1	38	0.699 (0.557–0.840)	23.5	34
Posterior thigh dominant leg	0.791 (0.691–0.891)	29.1	38	0.725 (0.593–0.857)	31	34
Posterior thigh nondominant leg	0.859 (0.788–0.930)	43.1	38	0.651 (0.494–0.809)	25.5	34
Posterior calf dominant leg	0.721 (0.595–0.847)	38.2	38	0.596 (0.422–0.769)	25.8	34
Calf nondominant leg	0.743 (0.624–0.861)	37.5	38	0.565 (0.384–0.747)	25.8	34
Indurometer						
Anterior thigh dominant leg	0.900 (0.848–0.952)	14.8	38	0.792 (0.055–0.901)	17.8	33
Anterior thigh nondominant leg	0.882 (0.821–0.942)	16.6	38	0.909 (0.857–0.961)	18.35	33
Posterior thigh dominant leg	0.925 (0.886–0.965)	32.3	38	0.869 (0.798–0.939)	24.6	34
Posterior thigh nondominant leg	0.956 (0.933–0.980)	31.3	38	0.942 (0.909–0.975)	27.2	34
Posterior calf dominant leg	0.942 (0.910, 0.973)	30.1	38	0.937 (0.901–0.972)	32.2	34
Calf nondominant leg	0.906 (0.857–0.955)	28.9	38	0.921 (0.877–0.965)	30.5	34
Mechanical Tonometer[a]						
Anterior thigh dominant leg	0.893 (0.838–0.949)	18.8	38			
Anterior thigh nondominant leg	0.906 (0.857–0.955)	11.6	38			
Posterior thigh dominant leg	0.952 (0.013–0.978)	19.7	38			
Posterior thigh nondominant leg	0.905 (0.856–0.955)	18.5	38			
Posterior calf dominant leg	0.964 (0.945–0.984)	20.4	38			
Calf nondominant leg	0.927 (0.888–0.965)	18.4	38			
SBF7						
Re dominant leg	>0.999 (>0.999–>0.999)	2.3	38	>0.999 (>0.999–>0.999)	14.3	32
Ri dominant leg	>0.999 (0.999–>0.999)	20.6	38	0.999 (0.999–>0.999)	27.7	32
Re nondominant leg	0.999 (0.998–0.999)	10.0	36	0.999 (0.998–0.999)	13.5	33
Ri nondominant leg	0.949 (0.920–0.978)	20.8	36	0.912 (0.863–0.962)	27.8	33

[a]Mechanical Tonometer used in the Myanmar group only.
ICC, intraclass correlation coefficient; CoV, coefficient of variation.

border indicate a higher ICC score (excellent agreement between repeated measures on a single measurement point) on a scale of 0–1.

The converse was true for the CoV. All devices recorded the lowest CoV (greatest agreement) at the anterior thigh. The relationship between agreements as represented by the CoV is demonstrated in Figure 2. Data points closer to the center represent less difference between the CoV (standardized SD as % of mean) of any device's measure at each point, on a scale between 0% and 40%.

Discussion

This study compared intrarater reliability of three tissue tonometers and one BIS device on the legs of young people in two tropical locations. It also compared agreement in the

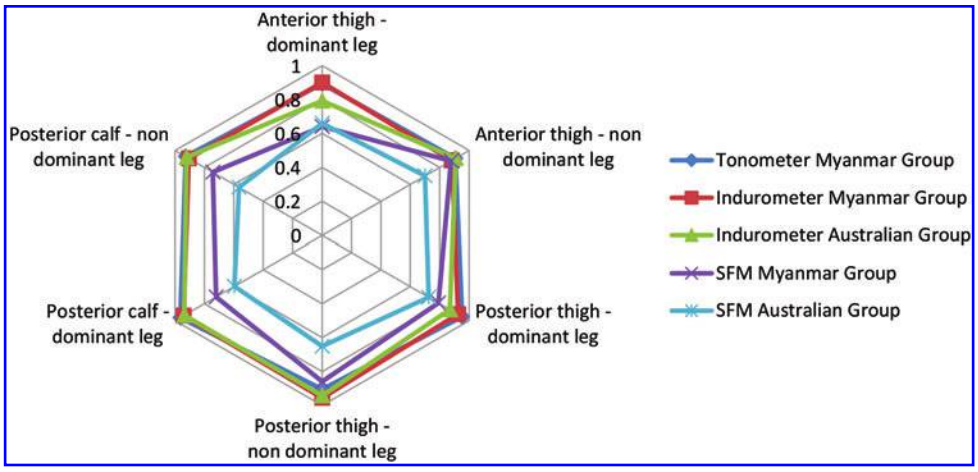

FIG. 1. Radar Graph of the ICC scores for the three tissue tonometers at six measuring points. ICC, intraclass correlation coefficient. A color version of this figure is available in the online article at www.liebertpub.com/lrb.

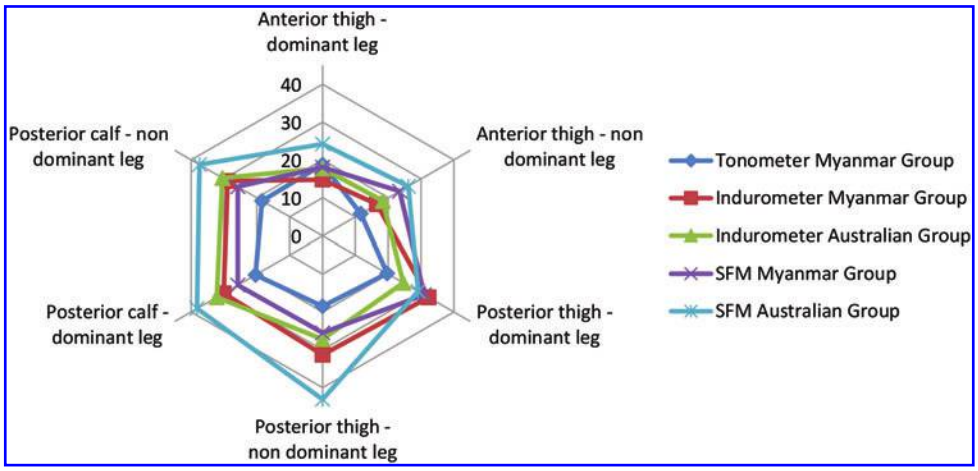

FIG. 2. Radar Graph of the CoV scores for the three tissue tonometers at six measuring points. CoV, coefficient of variation. A color version of this figure is available in the online article at www.liebertpub.com/lrb.

measurement rating scales between the three tissue tonometers: mechanical Tonometer, digital Indurometer, and SkinFibroMeter. BIS is an important tool in both clinical practice and research, is subject to very little operator influence, and, in this study, showed excellent reliability in keeping with multiple previous studies.[5,11,12,18,21] Mechanical tissue tonometers have also been important in quantifying fibrous changes associated with lymphedema that occur in the skin and underlying tissue, especially in research settings,[5,10] but their use in clinical settings has been hampered by problems with the device design, which can affect the measures.[13] The mechanism driving the indenter relies on gravity, so if the device is placed on a skin surface, which is not horizontal to the ground, the force of gravity will be changed and affect the true measure. Additionally, the analog readout must be viewed *in situ* and involves reading two dials, which record increments in different directions, that is, the whole millimeters are recorded on a small anticlockwise dial and the micrometer increments are recorded on a larger clockwise dial. Keeping the device horizontal and correctly reading the dials while also trying to reduce any parallax error can be cumbersome at some measurement sites. Furthermore, when soft edematous tissue is measured, the indenter continues to move slowly into the tissue and the dials may not come to a complete stop. The operator must then decide at what point to read the dials and this introduces a subjective component to the measure. This can be compounded when the first application leaves a visible indentation in the skin, thereby influencing subsequent measures at the same point.[13] Although the digital Indurometer was designed to overcome some of the limitations of the mechanical Tonometer, the comparative study by Vanderstelt et al.[14] found the CoV (%range) of measures with the newer device to be significantly higher (28.7%–33.6%) than the original mechanical unit (13.0%–20.2%) among participants with stage 1 BCRL. The differences were not significant at other measuring points (13.0%–33.6%) or when comparing measures on the control arms. The authors noted that although the Indurometer may not be as reliable as the mechanical Tonometer in the early fluid-rich stage of lymphedema, the ease of operation is a significant improvement and makes the device more accessible to a variety of operators. The SkinFibroMeter introduces a new set of parameters for tissue assessment and there are no previous reliability studies for comparison.

In our study on young healthy legs, there was little difference in ICC score between the mechanical Tonometer and Indurometer at any measuring point, and both devices demonstrated excellent reliability and low (good) CoV values. This is similar to the earlier study by Pallotta et al.[13] who reported a CoV (% range) for the mechanical Tonometer (8%–21%) and the Indurometer (7%–16%). Better scores in our study for the mechanical Tonometer (10.8%–20.4%) compared with the Indurometer (14.8%–32.3%) may be because the single operator had extensive experience using the mechanical Tonometer in research settings, whereas the previous studies used three[13] and seven operators.[14] However, both the digital devices have inbuilt sensors, which prevent any single measure from being recorded if the application is erratic, too fast/slow, or too heavy/light. They are designed to be used with minimal training and any influence of inexperience should have been quickly corrected during preliminary practice and familiarization sessions with negligible effect on the study results. It is more likely that the significant differences between devices found by Vanderstelt et al.[14] in stage 1 BCRL were related to the complex changes that occur during the onset and development of manifest lymphedema, particularly in the early fluid-rich stage when tissue changes are still in a state of flux and issues of indentation can confound accurate readings. This hypothesis is supported by the same study,[14] which found the lowest CoV values (greatest agreement) for measures of late-stage BCRL when fibrosis and thickened skin will be patently overt and less susceptible to fluctuation.

Conversely, in our study of healthy participants, greater variability (lower ICC score and higher %CoV) was found in measures of the harder skin of the calf rather than the softer tissue of the anterior thigh, so a fluid-rich tissue state is not the cause of variability in this cohort. Rather, it is likely that the poorer CoV values in calf measures were due to the size of the reference plate in relation to the anatomical location of the measure. The relatively large reference plates of both the mechanical Tonometer and the Indurometer often extended beyond the curve of the narrow calves of the children, whereas on the broader, softer thigh area, the reference plate could generally rest fully on the skin, even in smaller participants.

Similar issues with positioning the reference plate to fully contact the skin have also been reported during measures of the anterior chest wall.[13] Designers and manufacturers may wish to consider modification to a smaller reference plate for increased usability in nonuniform areas such as the anterior chest and for use with children and smaller limbs.

In the current study, the SkinFibroMeter showed excellent reliability in three of the measurement points in the Myanmar group and good reliability in all other measurement points in both groups, but when assessing agreement, the CoV was higher (poorer) for this device than the other tissue tonometers and also higher than the CoV reported in previous studies of tonometry. Additionally, the lowest (best) CoV for the SkinFibroMeter was for the anterior thighs (18.1%–26.1%) with higher (worse) CoV for the calves (25.8%–38.2%), which is opposite to the other two devices. It is difficult to understand what might account for the increased variability with this device since the smaller reference plate and inbuilt error rejection should make it more reliable in narrow limbs. The very light technique may make it more difficult to apply the reference plate evenly, or it may be more susceptible to pressure variation by the operator, and the range at which the error measures are rejected is not known.

All three tissue tonometers recorded the softest absolute measures at the midpoint of the anterior thigh where the fatty layer over the muscle would naturally be thicker than the skin over the musculotendinous junction, which lies at the midpoint of the calf. The latter showed the hardest measures, with values over the more muscular posterior thigh falling between the two. BIS measures indicated a slightly higher fluid load in the nondominant leg in both young populations, which is opposite to reports of slightly increased fluid loads in the dominant arms of normal adults.[8,15]

Although all four devices were found to be reliable, the level of reliability as indicated by the ICC scores was related to the type or composition of the underlying tissue at the measurement point. These differences may be small and not a direct indication of reliability in lymphedema, but they have the potential to impact results. Limb dominance and the anatomical features of the measurement location should be taken into account when choosing the best tool for the measure and when interpreting measures that compare affected and unaffected limbs in lymphedema.

Conclusions

The findings of this study that all four devices have good to excellent reliability have widened the demographic in which they can be used to include the lower limbs of a young nonlymphedema population. Given the operational issues associated with the mechanical Tonometer and based on usability, reliability, and agreement between devices, the newer digital Indurometer is an acceptable replacement for the mechanical Tonometer to measure tissue compressibility in young people without lymphedema. The SkinFibroMeter is also reliable and shows good agreement between tissue tonometers in this group, although to a slightly lesser degree than the other two tonometers. All four devices are appropriate for use in future studies on the legs of young people without clinical signs of lymphedema. Further evaluation of the newer digital devices in lymphedema populations is needed and in particular studies on leg lymphedema are missing from the literature.

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Author Disclosure Statement

No competing financial interests exist for any of the authors. The mechanical Tonometer and Indurometer used were owned by J.C.U. and the SkinFibroMeter was loaned by the manufacturer at no charge and without influence over the study design, analysis, or reporting of results. J.C.U. has no financial interest in the results of this study.

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Supplementary Data

Supplementary Table S1. Absolute Values of Tonometer, Indurometer, and SkinFibroMeter at All Measuring Points and Absolute Values of BIS for Each Leg

	Myanmar n = *38*				*Australia* n = *34*			
Measuring point	*Dominant leg*	N	*Nondominant leg*	n	*Dominant leg*	n	*Nondominant leg*	n
SFM (Newtons) mean (SD)								
Anterior thigh	0.062 (0.015)	38	0.069 (0.018)	38	0.072 (0.013)	34	0.068 (0.016)	34
Posterior thigh	0.086 (0.025)	38	0.072 (0.031)	38	0.087 (0.027)	34	0.106 (0.027)	34
Calf	0.102 (0.039)	38	0.088 (0.033)	38	0.124 (0.032)	34	0.124 (0.032)	34
Indurometer (0.01 mm) mean (SD)								
Anterior thigh	4.677 (0.690)	38	4.558 (0.757)	38	5.171 (0.922)	33	5.069 (0.937)	33
Posterior thigh	3.509 (1.134)	38	3.612 (1.129)	38	3.964 (0.976)	34	3.740 (1.018)	34
Calf	2.158 (0.649)	38	2.235 (0.645)	38	2.673 (0.856)	34	2.791 (0.850)	34
Mechanical Tonometer (0.1 mm) mean (SD)								
Anterior thigh	7.316 (0.790)	38	7.223 (0.835)	38	n/a		n/a	
Posterior thigh	5.850 (1.150)	38	5.917 (1.095)	38	n/a		n/a	
Calf	4.154 (0.849)	38	4.295 (0.791)	38	n/a		n/a	
BIS (Ohms) mean (SD)								
Ri (ICF)	885.042 (182.176)	38	901.743 (187.598)	36	776.395 (214.923)	32	786.724 (218.910)	33
Re (ECF)	357.249 (8.338)	38	366.180 (36.74)	36	325.807 (46.674)	32	327.866 (44.407)	33
Ri:Re	2.393	38	2.463	36	2.383	32	2.400	33

n/a, data not available; BIS, bioimpedance spectroscopy; ECF, extracellular fluid; ICF, intracellular fluid; SD, standard deviation.

Appendix F3: Moderating factors in device measures

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Title: Moderating factors in tissue tonometry and bio-impedance spectroscopy measures in the lower extremity of healthy young people in Australia and Myanmar.

Abbreviated title: IND TON SFM BIS healthy young legs Aust & Myanmar

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Abstract

Background: Tissue tonometry and bio-impedance spectroscopy (BIS) are commonly used to assess changes occurring in breast cancer-related lymphedema of the arm. Expected values in the lower extremity of young people have not been established. This study investigated expected values and factors contributing to variation in tissue compressibility and free fluid in two tropical populations. **Methods and Results:** A convenience sample of healthy young volunteers aged 8 - 21 in Myanmar and Australia without history of lymphedema or lower limb injury. Data was collected about possible modifiers; leg dominance, age, gender, body mass index (BMI), hydration, and menstrual cycle. Tissue compressibility at the anterior thigh, posterior thigh and calf was measured using a Digital Indurometer, Mechanical Tonometer and SkinFibrometer. Free fluid in each leg was assessed using BIS. Paired t-test and linear regression compared the objective measures with possible modifiers within each population. Statistical significance was set at $p<0.05$ with a 95% confidence interval. In Myanmar increases in free fluid, tissue compressibility and limb circumference were associated with being older, female, underweight or in the second half of the menstrual cycle. In young Australians, increases in tissue compressibility and limb circumference were associated with being older or in the second half of the menstrual cycle. **Conclusion:** When assessing tissue compressibility and free fluid in young people using tonometry and BIS, limb dominance and BMI should be considered in a local context and attempts should be made to minimize the potential influences of hydration and the female menstrual cycle.

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Condensed Abstract

Lymphatic Filariasis (LF) is the largest cause of lymphedema worldwide, yet devices commonly used to assess lymphedema in developed countries have not been applied in LF endemic settings. This study presents a cross-sectional analysis of tissue compressibility and free fluid measures in the legs of health young people living in tropical settings in Myanmar and Australia. Leg dominance, body composition, gender, hydration and the menstrual cycle were all associated with variation in measures. Where possible these moderating factors should be considered and minimised when using these devices in these populations.

Key words:

Tonometry Methods

Electric Impedance

Body Composition

Lower Extremity

Geographic Factor

Introduction

The lymphatic system collects circulating proteins and fluid from the tissue spaces and returns them to the cardiovascular system. If lymphatic function is disrupted a high protein edema accumulates around the distal lymphatics under the skin and is termed lymphedema. Untreated lymphedema follows a predictable course of changes in the skin and underlying tissue. In the earliest stages, the edema is fluid and protein rich, the skin is soft to touch and there is minimal or transient visible swelling. In the middle stages, the subcutaneous compartment visibly enlarges and slowly becomes filled with fat and fibrous tissue which ultimately replaces the edematous tissue spaces, there is increased vascularisation and free fluid reduces. In later stages the skin becomes thickened, the tissue feels hard on palpation and there is little or no excess free fluid. This spectrum of changes can be measured objectively using tissue tonometry to quantify changes in skin and subcutaneous tissue stiffness, bio-impedance spectroscopy to detect increases in extracellular free fluid load and a tape measure for changes in limb circumference.[1 2] These devices have been validated in research on breast cancer related lymphedema (BCRL) of the arm, largely among post-menopausal women in affluent countries after unilateral intervention for breast cancer where the contralateral 'unaffected' limb is available as a within subject control [1-3]. But lymphedema is a disease of many causes, affects all age groups and genders and is influenced by environmental, familial and genetic factors. When both legs are involved there is no unaffected, control limb and even when only one leg is involved the 'unaffected' leg may be in a state of covert or latent lymphedema. In either case the contralateral leg is not a reliable control and there is little data available on lymphedema of the lower limb or in young people.

Studies in unaffected adults and children have shown that age, gender and body mass index

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(BMI) all influence BIS measures [4, 5] and it is likely that these same factors will influence tissue compressibility but no corresponding data is available to support this. The World Health Organization (WHO) advises that normal BMI for adults in Asian countries can be 3kg/m^2 less than Western standard [6] and growth data from developed countries may not be applicable in developing country settings [7]. Systemic hydration, hormonal changes during the menstrual cycle and local muscle mass will all affect extracellular free fluid and therefore should also be considered.

Globally, 16 million people have lymphedema in one or both the lower limbs after infection with lymphatic filariasis (LF), a parasitic disease closely associated with poverty in developing countries [8]. In preparation for a study to detect early lymphatic change in young people living in a filariasis endemic area in Myanmar, limb circumference, tissue tonometry and BIS data were collected on two sets of young people living in tropical settings. Intra-rater reliability analysis of the devices was good to excellent in these populations [9] and mean values reported for these healthy young cohorts may be used to identify cut off points in similar population groups with clinical signs of lymphedema or as control data in clinical trials.

Materials and Methods

This was a cross sectional study on physical measures of the lower extremity in two tropical dwelling cohorts of healthy young people. All procedures were conducted in accordance with the Helsinki Declaration of 1975, as revised in 2008. The James Cook University Human Research Ethics Committee (approval numbers H5261 and H5497) and the Department of Health, Myanmar Ministry of Health approved this study. Young people aged 18 – 21 years

gave informed consent to participate and a parent or guardian of minors aged 8 – 17 years provided consent and was asked to remain with the child during all data collection. Verbal assent was reiterated before any measure was taken. Volunteers were excluded if they had any clinical sign of lymphedema or any injury to their lower limbs. Participants were asked 'Which foot do you use to kick a ball?' to determine leg dominance. Time since the last drink was used as a proxy for hydration and young women were asked the number of days since their last menses. Data was collected between October 2014 and May 2015 and in Myanmar only persons who tested negative for LF infection were included in this analysis.

In Myanmar, local research assistants interviewed the participants, measured height and weight and completed all data collection sheets. In Australia the participant, their guardian or a peer filled in the data collection sheets. The principal researcher (JD) interviewed the Australian participants and applied all tonometers in both locations. Tissue compressibility was measured at the same mid-points with the addition of the mid-posterior thigh. Tissue compressibility was assessed using three tissue tonometers; a Digital Indurometer and a Mechanical Tonometer which both measure tissue compliance to a 200gm force and a SkinFibrometer which measures skin resistance to 50gms. All three tonometers were used in the Myanmar group but only the two digital devices were used in the Australian group. Measures w3ere taken at the midpoint of the anterior thigh, posterior thigh and calf of each leg. Whole limb free fluid in both cohorts was assessed using BIS to measure electrical impedance in the intracellular fluid (ICF) and extracellular fluid (ECF) compartments and this is then recorded as a ratio of resistance ICF to resistance ECF (Ri:Re). Circumference data were collected at midpoint of the anterior thigh and posterior calf. More detailed data

collection methods were previously published in a reliability study on the devices in the same populations [9].

Data analysis

An online calculator was used to estimate a sample size of 32 subjects per group using an expected mean Digital Indurometer value of 2.5 (SD 0.7) at mid-calf [9] with a 95% confidence interval and 80% power [a]. Height and weight data were used to calculate BMI using the formula; kg/m^2. Growth charts for adolescents in the USA were used to identify underweight (in or below the 5th percentile) or overweight (in or above 85th percentile) participants. Charts were accessed online during June 2016 [b]. The effect of age was evaluated by dividing participants into younger (8 – 16 years) or older (17 – 21 years) age groups. In Australia the average of three measures was recorded for all devices. In the Myanmar cohort, an average of either three or two measures was used dependent on whether the participant had been included in a previous reliability study of the devices (3 measures) [9] or not (2 measures). Between-country comparisons were made using independent samples T-tests for continuous variables, Chi-Square tests were used for categorical variables reporting p values for Fishers exact test and paired samples T-tests were used to compare outcome measures in dominant and non-dominant legs. Linear regression was used to determine how moderating factors were associated with variance in scores. The regression was repeated to produce a model with as few variables as possible by removing one insignificant factor at a time. Only factors which significantly predicted variance in scores have been reported. All analysis was completed using SPSS Version 23.

Footnotes

a) http://biomath.info/power/ttestnoninf.htm

b) https://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.html

Results

The study sites of Amarapura (near Mandalay, Myanmar) and Townsville (Queensland, Australia) are approximately equidistant from the equator and both locations have dry tropical climates. All the Australian volunteers and the uninfected participants in Myanmar were included in this analysis (n=85). Table 1 shows the characteristics of the Myanmar and Australian cohorts. The mean age was 15.18 years (SD 3.66, range 8 - 21) and there were more females in each cohort: 57% in Myanmar and 60% in Australia. Mean height was 155.43cm (SD 13.15, range 118.80 - 187.50cm) and mean weight was 46.5kg (SD 13.7, range 17.5 - 86.1kg). Australian participants were significantly taller and heavier than the Myanmar cohort and did not differ markedly from US anthropometric data. A greater portion of Myanmar participants were in or below the 5th percentile for BMI, n=16 (31.4%), compared to Australians, n= 3 (8.8%) but this was only statistically significant between the male cohorts. There were no overweight cases in either group and both cohorts were right leg dominant, Myanmar 98% and Australia 94%. A greater portion of the Myanmar cohort had less recent hydration than their Australian counterparts (73.5% vs 39.4%) (defined as not drank within the hour prior to being measured). There were no significant between country differences in age groups or time since the last menstrual period (females).

Devices, measuring points and leg dominance

A consistent pattern of most compressible (softest) tissue over the anterior thighs and least compressible (hardest) tissue at the calves was found with all three tonometers. Values for the posterior thighs fell between the two and this pattern held true for of age, gender and country subgroups. There was statistically more free fluid in the dominant leg (p=0.034) but the between leg difference was small (7%) and this pattern did not hold true for subgroups by country and gender. In both cohorts being in the older age group was associated with larger

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Table 1: Comparison of participant characteristics in Myanmar and Australian cohorts.

Characteristic	Gender	Myanmar n=51	Australia n=34	p=
Aged 17 – 21 years n= (%)	Male	6 (27.3%)	5 (35.7%)	0.604
	Female	13 (44.8%)	9 (45.0%)	0.991
Height in cm mean (SD)	Male	151.40 (13.97)	158.96 (20.97)	0.189
	Female*	152.88 (9.13)	161.08 (9.03)	0.003
Weight in kg mean (SD)	Male*	40.77 (10.39)	51.92 (20.27)	0.037
	Female*	43.40 (9.67)	53.38 (12.94)	0.003
BMI kg/m^2 mean (SD)	Male*	17.46 (2.41)	19.62 (3.42)	0.033
	Female*	18.43 (2.83)	20.29 (3.31)	0.041
BMI ≤ 5th percentile^ n= (%)	Male*	12 (54.5%)	1 (7.1%)	0.004
	Female	4 (13.8%)	2 (10.0%)	0.527
Last drink > 1 hour before n= (%)	Male	13 (59.1%)	5 (35.7%)	0.194
	Female*	23 (79.3%)	8 (40.0%)	0.002
Last menses < 14 days prior n= (%)	Female	10 (34.5%)	8 (40.0%)	0.672

*significant between group differences
BMI=body mass index
^CDC growth charts for children in USA
https://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.htm

limb circumference and being underweight was associated with smaller limb circumference. In the Myanmar cohort, the skin was less compressible over the anterior thigh muscles on the dominant side and thigh circumference was larger on that side. There was skin was less compressible over the posterior thigh and calf in the non-dominant leg, the calf circumference was larger and there was slightly more free fluid in that leg. In the Australian cohort, the circumference of the thigh and calf were both slightly larger in the dominant leg with significantly more free fluid on that side but no between limb pattern of tissue compressibility. Mean values and between limb differences for Myanmar and Australian cohorts are provided in Supplementary Table A.

SkinFibroMeter

In the Myanmar cohort, higher tissue compressibility was associated with being in the older age group (17-21 years) at the dominant anterior thigh and with being female or in the second half of the menstrual cycle will all measures. Lower compressibility values at both calves were associated with less recent hydration (not having a drink in the previous hour). In the Australian cohort higher compressibility at the non-dominant anterior thigh and both posterior thighs was associated with being in the older age group. Being female was associated with less compressibility at the non-dominant anterior thigh. Significant factors associated with SkinFibroMeter measures are given in Table 2.

Digital Indurometer

In the Myanmar cohort, higher tissue compressibility at the dominant anterior thigh was associated with being in the older age group. For all other measures being female was associated with more compressibility and less recent hydration was associated with less tissue compressibility. Being underweight was associated with increased compressibility at the non-dominant calf. In the Australian cohort, increased tissue compressibility at both posterior

Table 2: Factors significantly associated with variation in measures using the SkinFibroMeter

Country/ measure	Factor	Unstandardised Coefficient (95%CI)	Direction	t=
Myanmar				
Anterior thigh				
Dominant	17 – 21 years	-0.009 (-0.016, -0.002)	Softer	-2.607*
	Female	-0.012 (-0.018, -0.005)	Softer	-3.455**
Non-dominant	>14 days since menses	-0.010 (9-0.014, -0.006)	Softer	-4.972**
Posterior thigh				
Dominant	Female	-0.026 (-0.036, -0.016)	Softer	-5.151**
Non-dominant	Female	-0.019 (-0.032, -0.007)	Softer	-3.109**
Calf				
Dominant	Female	-0.024 (-0.041, -0.006)	Softer	-2.746**
	> 1hr since last drink	+0.024 (0.004, 0.043)	Harder	2.432*
Non-dominant	Female	-0.024 (-0.041, -0.007)	Softer	-2.817**
	> 1hr since last drink	+0.019 (0.000, 0.038)	Harder	1.972*
Australia				
Anterior thigh				
Non-dominant	17 – 21years	-0.011 (-0.020, -0.002)	Softer	-2.557*
	Female	0.012 (0.003, 0.020)	Harder	2.642*
Posterior thigh				
Dominant	17 – 21years	-0.020 (-0.036, -0.004)	Softer	-2.559*
Non-dominant	17 – 21years	-0.021 (-0.036, -0.005)	Softer	-2.720*

* $p < 0.05$
** $p < 0.01$

thighs was associated with being in the older age group or underweight and at both calves with being underweight. Significant factors associated with Digital Indurometer measures are given in Table 3.

Mechanical Tonometer

Used only in the Myanmar group, higher tissue compressibility at both anterior thighs was associated with being in the 17 – 21 age group and at all posterior leg measures with being female or in the second half of the menstrual cycle. Less recent hydration was associated with less compressibility at all posterior leg measures. Table 4 shows factors significantly associated with Mechanical Tonometer measures in the Myanmar group.

SBF7

In Myanmar, less recent hydration was associated with less free fluid in both legs whereas increased free fluid was associated with being the older age group (dominant leg) or in the second half of the menstrual cycle (non-dominant leg). There were no significant associations between any of the factors and BIS measures in the Australian group. Factors significantly associated with BIS measures are given in Table 5.

Circumference

In addition to expected variances associated with age or being underweight, being in the second half of the menstrual cycle was associated with larger circumference measures at the dominant thigh in the Myanmar group and with all circumference measures in the Australian group. Less recent hydration was associated with smaller circumference at the dominant thigh in the Myanmar group. Significant factors associated with limb circumference are given in Table 6.

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Table 3: Factors significantly associated with variation in measures using the Digital Indurometer

Country/ measure	Factor	Unstandardised Coefficient (95%CI)	Direction	t=
Myanmar				
Anterior thigh				
Dominant	17 – 21 years	0.636 (0.276, 0.996)	Softer	3.548**
Non-dominant	Female	0.424 (0.039, 0.810)	Softer	2.216*
	> 1hr since last drink	-0.420 (0.852, 0.013)	Harder	-1.954*
Posterior thigh				
Dominant	Female	0.866 (0.419, 1.312)	Softer	3.900**
	> 1hr since last drink	-0.668 (-1.169, -0.167)	Harder	-2.685*
Non-dominant	Female	0.962 (0.483, 1.441)	Softer	4.042**
	> 1hr since last drink	-0.777 (-1.314, -0.240)	Harder	-2.912**
Calf				
Dominant	Female	0.781 (0.448, 1.113)	Softer	4.725**
	> 1hr since last drink	-0.419 (-0.792, -0.046)	Harder	-2.263*
Non-dominant	Female	0.898 (0.559, 1.237)	Softer	5.331**
	> 1hr since last drink	-0.606 (-0.954, -0.257)	Harder	-3.499**
	< 5th percentile for BMI	0.526 (0.169, 0.882)	Softer	2.970**
Australia				
Posterior thigh				
Dominant	17 – 21 years	0.788 (0.215, 1.360)	Softer	2.804**
	< 5th percentile for BMI	1.281 (0.287, 2.274)	Softer	2.628*
Non-dominant	17 – 21 years	1.126 (0.559, 1.692)	Softer	4.054**
	< 5th percentile for BMI	1.220 (0.237, 2.202)	Softer	2.531*
Calf				
Dominant	< 5th percentile for BMI	1.583 (0.635, 2.531)	Softer	3.400**
Non-dominant	< 5th percentile for BMI	1.103 (0.137, 5.068)	Softer	2.326*

* $p < 0.05$
** $p < 0.01$

Table 4: Factors associated with variation in measures using the Mechanical Tonometer (Myanmar Cohort)

Country/ measure	Factor	Unstandardised Coefficient (95%CI)	Direction	t=
Anterior thigh				
Dominant	17 – 21 years	0.921 (0.466, 1.376)	Softer	4.065**
Non-dominant	17 – 21 years	0.504 (0.056, 0.953)	Softer	2.264*
	Female	0.538 (0.101, 0.976)	Softer	2.474*
Posterior thigh				
Dominant	> 1hr since last drink	-0.891 (-1.494, -0.288)	Harder	-2.976**
	>14 days since menses	0.883 (0.541, 1.226)	Softer	5.197**
Non-dominant	Female	0.998 (0.484, 1.511)	Softer	3.911**
	> 1hr since last drink	-0.895 (-1470, 0.319)	Harder	-3.129**
Calf				
Dominant	Female	1.290 (0.844, 1.736)	Softer	5.824**
	> 1hr since last drink	-0.674 (-1.174, -0.174)	Harder	-2.715**
Non-dominant	Female	1.042 (0.629, 1.456)	Softer	5.077**
	> 1hr since last drink	-0.636 (-1.099, -0.172)	Harder	-2.762**

* $p < 0.05$
** $p < 0.01$

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Table 5: Factors associated with variation in measures using BIS in the Myanmar cohort.

Limb	Factor	Unstandardized Coefficient (95%CI)	Direction	t=
Dominant leg	17 – 21 years	-0.342 (-0.575, -0.108)	More	-2.295**
	> 1hr since last drink	0.415 (0.172, 0.658)	Less	3.445**
Non-dominant leg	> 1hr since last drink	0.329 (0.046, 0.612)	Less	2.347*
	>14 days since menses	-0.253 (-0.420, -0.087)	More	-3.065**

* $p < 0.05$
** $p < 0.01$

Table 6: Factors significantly associated with variation in measures of limb circumference.

Country/ measure	Factor	Unstandardised Coefficient (95%CI)	Direction	t=
Myanmar				
Thigh				
Dominant	17 – 21 years	5.199 (2.625, 7.773)	Larger	4.071**
	< 5th percentile for BMI	-5.244 (-7.869, -2.619)	Smaller	-4.026**
	> 1hr since last drink	-2.779 (-5.318, -0.239)	Smaller	-2.205*
	>14 days since menses	1.770 (0.827, 0.238)	Larger	-2.140*
Non-dominant	17 – 21 years	6.444 (4.190, 8.698)	Larger	5.749**
	< 5th percentile for BMI	-6.470 (-8.819, -4.122)	Smaller	-5.540**
Calf				
Dominant	17 – 21 years	2.577 (1.348, 3.806)	Larger	4.217**
	< 5th percentile for BMI	-3.051 (-4.332, -1.771)	Smaller	-4.791**
Non-dominant	17 – 21 years	2.317 (1.113, 3.521)	Larger	3.869**
	< 5th percentile for BMI	-2.936 (-4.191, -1.682)	Smaller	-4.706**
Australia				
Thigh				
Dominant	17 – 21 years	6.746 (2.944, 10.547)	Larger	3.629**
	< 5th percentile for BMI	-11.562 (-17.374, -5.750)	Smaller	-4.069**
	>14 days since menses	2.917 (0.723, 5.110)	Larger	2.720*
Non-dominant	17 – 21 years	6.800 (3.169, 10.430)	Larger	3.830**
	< 5th percentile for BMI	-11.689 (-17.240, -6.137)	Smaller	-4.306**
	>14 days since menses	2.794 (0.699, 4.889)	Larger	2.728*
Calf				
Dominant	17 – 21 years	3.662 (1.458, 5.866)	Larger	3.398**
	< 5th percentile for BMI	-4.973 (-8.343, -1.604)	Smaller	-3.018**
	>14 days since menses	1.509 (0.237, 2.780)	Larger	2.426*
Non-dominant	17 – 21 years	4.033 (1.819, 6.248)	Larger	3.726**
	< 5th percentile for BMI	-5.270 (-8.656, -1.885)	Smaller	-3.184**
	>14 days since menses	1.369 (0.091, 2.646)	Larger	2.191*

* $p < 0.05$
** $p < 0.01$

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Healthy, prepubescent children in all countries will undergo somewhat predictable changes in body size and composition as they transition through adolescence and become young adults. In keeping with this expectation, limb circumference in both cohorts increased in an unremarkable association with age while being underweight was associated with smaller limb circumferences and softer (less muscular) tissues. There were small but significant directional changes associated with limb dominance in most measures. In the Myanmar cohort, the kicking leg, labelled as the dominant leg, had a slightly larger thigh circumference while the other (non-dominant) leg, which supports the weight of the body and propels it forward during the kick, had a slightly larger calf circumference. Trends in tissue compressibility support this pattern of muscle development as does the lack of any real between-leg difference in free fluid loads, which together suggest that neither lower limb is more developed overall than the other in this cohort. In a similar study conducted in Papua New Guinea (PNG) on a comparatively aged group of young people, a similar pattern of leg circumference (larger dominant thigh, larger non-dominant calf) was reported [10]. In contrast, the Australian cohort appeared to be more homolateral, with slightly larger thigh and calf circumferences and more free fluid all on the dominant side. In healthy humans, homeostasis will ensure extracellular fluid loads are maintained within a narrow range of normal and accordingly we found only minimal differences in free fluid ratios between legs and similar free fluid loads as reported in the PNG study. The lack of any clear cross-cultural consistency in between leg differences in any measure indicates that a universal assumption for leg dominance cannot be made.

When considering compressibility measures, the fatty layers over the anterior thighs were the most compressible (softest) whilst the mid-calf point, located over a dense tendo-muscular

junction, were always the least compressible (hardest). Mechanical Tonometry (which was used in both the Myanmar and PNG groups) found softer tissues in the Myanmar group, while the Digital Indurometer (which was used in the Myanmar and Australian groups) found softer tissues in the Australian group. These two devices do not return equivalent raw values but are essentially measuring the same thing. The direction of association between location - PNG, Myanmar, Australia - and increasingly softer tissues suggests that higher tissue compressibility is associated with living in more economically developed environments. If this is true, the cut off figures proposed to identify subclinical change between LF infected and uninfected young people in the PNG study [10] should not be generalised to other populations. Rather, expected patterns of normal musculature and tissue compressibility should be viewed in a local context.

Where a regression model indicated one or more significant variances with any device, the factor variable was often age, gender, hydration or menstrual cycle. Where age or gender was a factor, being older, female or in the second half of the menstrual cycle, was always associated with a larger, softer limb with more free fluid. The effects of being female were more frequently found in the Myanmar cohort and although not universal, suggest that small fluctuations during the menstrual cycle of young women should be considered when using all devices. With less than 30% of the Myanmar cohort consuming a drink within the hour before the measures it is perhaps not surprising that this factor was associated with a smaller limb circumference, lower tissue compressibility and less free fluid at almost every measure in this group. The better hydrated Australian cohort showed almost no association between hydration and device measures. This effect of hydration could be easily controlled by administering a standardized drink prior to measures being recorded.

There are no universal guidelines for detection of early pathology in lymphatic function, especially in the lower limb, but considering the small variances found for many measures, not all factors need to be considered as potential confounders to assessment. Never-the-less there are some device- and country-specific factors that should be considered. When using the Digital Indurometer age and BMI should be always be considered. In the Myanmar population, young females can be expected to return softer values than males using any tonometer.

The study was limited by an inability to control for ambient temperature but the study sites were roughly equidistant from the equator in dry tropical zones with similar climates. Data was collected in a village administration centre in Myanmar without air-conditioning and in an air-conditioned office in Australia and although ambient temperatures and humidity were not factored into the analysis, conditions were representative of usual health care environments in each location. The single operator could not be adequately blinded to any of the parameters measured but the use of a scribe reduced the risk of bias. The CDC growth charts will most likely have misclassified several young Myanmar people of normal BMI to the underweight category; therefore variances associated with being underweight should be viewed cautiously. The sample was large enough to provide statistical significance for the between leg differences and the regression analysis but recruitment methods prevent the data from being truly representative of each population. Nevertheless, mean values, standard deviations and regression models provided may be useful in estimating expected values and cut-off points in future lymphatic research on young populations.

Conclusion

This study has demonstrated country specific effects of age, gender, BMI, hydration and menstrual cycle on tissue compressibility and free fluid loads in the lower limbs of young people. The definition of leg dominance and normal BMI should be determined in the context of local life. Other moderating factors could be minimised by administering a drink during the hour prior to measures and by collecting data on young women in the first half of the menstrual cycle.

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Disclosure Statement

This study was conducted as part of a longitudinal study on early detection of lymphatic dysfunction in Myanmar and undertaken as a part of the PhD research project of Janet Douglass. No competing financial interests exist.

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Supplementary Table A: Mean (SD) values and between-limb differences for each measure using tissue tonometry, bio-impedance spectroscopy and limb circumference in a) Myanmar and b) Australia.

a) Myanmar							
	N=	**Dominant Mean (SD)**	**Non-dominant Mean (SD)**	**Mean difference (SD)**	**95% CI**	**% difference**	**t=**
SkinFibroMeter							
Anterior thigh	51	0.072 (0.014)	0.067 (0.014)	0.005 (0.011)	0.002,0.008	6.9	3.263**
Posterior thigh	51	0.084 (0.022)	0.084 (0.024)	0.005 (0.006)	0.166,0.000	0.0	0.021
Calf	51	0.096 (0.032)	0.106 (0.030)	0.017 (0.003)	-2.807,-0.010	17.7	0.026**
Digital Indurometer							
Anterior thigh	51	4.725 (0.687)	5.030 (0.704)	-0.305 (0.496)	-0.445,-0.166	6.5	-4.397**
Posterior thigh	51	4.074 (0.862)	3.884 (0.948)	0.190 (0.568)	0.031,0.350	4.7	2.396*
Calf	51	2.718 (0.663)	2.485 (0.660)	0.233 (0.359)	0.132,0.334	8.6	4.635**
Mechanical Tonometer							
Anterior thigh	51	6.551 (0.896)	7.105 (0.841)	-0.554 (0.699)	-0.751,-0.358	8.5	-5.661**
Posterior thigh	51	5.589 (1.180)	5.576 (0.997)	0.013 (0.791)	-0.210,0.235	0.0	0.114
Calf	51	4.243 (0.957)	4.024 (0.871)	0.219 (0.587)	0.054,0.384	5.2	2.664**
BIS							
Ri:Re	48	2.542 (0.447)	2.501 (0.479)	0.038 (0.602)	-0.145,0.221	1.5	0.422
Circumference							
Calf	51	24.739 (2.746)	24.765 (2.639)	-0.250 (0.667)	-0.213,0.162	1.0	-0.273
Thigh	51	42.510 (5.732)	42.184 (5.623)	0.325 (0.927)	0.065,0.586	0.8	2.508*

b) Australia							
	N=	**Dominant Mean (SD)**	**Non-dominant Mean (SD)**	**Mean difference (SD)**	**95% CI**	**% difference**	**t=**
SkinFibroMeter							
Anterior thigh	34	0.072 (0.012)	0.068 (0.014)	0.004 (0.012)	-0.001,0.008	5.0	1.531
Posterior thigh	34	0.087 (0.024)	0.106 (0.024)	-0.019 (0.021)	-0.026,-0.012	21.8	-5.335**
Calf	34	0.124 (0.027)	0.124 (0.027)	0.000 (0.030)	-0.010,0.011	0.0	0.095
Digital Indurometer							
Anterior thigh	34	5.171 (0.862)	5.069 (0.917)	0.103 (0.751)	-0.168,0.373	2.0	0.773
Posterior thigh	34	3.964 (0.941)	3.740 (1.008)	0.225 (0.486)	0.055,0.394	5.7	2.696*
Calf	34	2.692 (0.884)	2.791 (0.835)	-0.099 (0.498)	-0.273,0.075	3.7	-1.156
BIS							
Ri:Re	31	2.354 (0.444)	2.841 (0.643)	-0.487 (0.760)	-0.766,-0.209	20.7	-3.571**
Circumference							
Calf	34	28.097 (4.002)	28.024 (4.093)	0.074 (0.444)	-0.081,0.228	0.3	0.966
Thigh	34	48.112 (7.553)	47.726 (7.417)	0.385 (0.817)	0.100,0.670	0.8	2.749**

* $p < 0.05$
** $p < 0.01$
BIS = bio-impedance spectroscopy
Ri:Re = ratio intracellular to extracellular resistance

Appendix F4: Cross-sectional analysis – Myanmar

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Article

Lymphatic Filariasis Increases Tissue Compressibility and Extracellular Fluid in Lower Limbs of Asymptomatic Young People in Central Myanmar

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Abstract: When normal lymphatic function is hampered, imperceptible subcutaneous edema can develop and progress to overt lymphedema. Low-cost reliable devices for objective assessment of lymphedema are well accepted in clinical practice and research on breast-cancer related lymphedema but are untested in populations with lymphatic filariasis (LF). This is a cross-sectional analysis of baseline data in a longitudinal study on asymptomatic, LF antigen-positive and -negative young people in Myanmar. Rapid field screening was used to identify antigen-positive cases and a group of antigen-negative controls of similar age and gender were invited to continue in the study. Tissue compressibility was assessed with three tissue tonometers, and free fluids were assessed using bio-impedance spectroscopy (BIS). Infection status was confirmed by Og4C3 antigen assay. At baseline (n = 98), antigen-positive cases had clinically relevant increases in tissue compressibility at the calf using a digital Indurometer (11.1%, p = 0.021), and in whole-leg free fluid using BIS (9.2%, p = 0.053). Regression analysis for moderating factors (age, gender, hydration) reinforced the between-infection group differences. Results demonstrate that sub-clinical changes associated with infection can be detected in asymptomatic cases. Further exploration of these low-cost devices in clinical and research settings on filariasis-related lymphedema are warranted.

Keywords: neglected tropical disease; lower extremity; lymphatic filariasis; tissue tonometry; bio-impedance spectroscopy; lymphedema

1. Introduction

Lymphatic filariasis (LF) is a parasitic disease in which thread-like worms inhabit the human lymphatic system, where they can impair normal lymphatic pumping. Classified as a neglected tropical disease and affecting many of the world's poorest populations, LF can lead to lymphedema, a progressively debilitating swelling of the skin and subcutaneous tissue in any body part, most frequently the legs [1]. Normal lymphatic pumping actively removes circulating proteins and fluid from the tissue spaces, maintaining a slightly negative interstitial pressure. When lymphatic capacity

is impaired, extracellular fluid (ECF) and circulating proteins begin to accumulate in the interstitial spaces [2]. If normal lymphatic function is not restored, this initially covert edema gradually becomes overt, and the affected body part visibly enlarges. Over time, the protein-rich fluid is replaced with fat and fibrous tissue, and normal limb contours are lost. The outdated eponym 'elephantiasis' was inspired by the appearance of a grossly enlarged limb in late-stage lymphedema where the skin is thick, discolored, and formed into folds. In developed countries, lymphedema is frequently caused by surgical damage when lymph nodes are removed or irradiated during cancer treatment. Much of what is known about initiation and progression of lymphedema comes from research on breast cancer-related lymphedema (BCRL) of the arm [3].

A wide spectrum of devices and methods is used to objectively evaluate lymphedema depending on the setting. At the highly resourced end of the spectrum, nuclear imaging and other sophisticated technologies are often used to assess BCRL of the arm. Tissue tonometry to quantify tissue compressibility and portable bio-impedance spectroscopy (BIS) to track fluctuations in free fluid are also used and are relatively inexpensive. Using BIS, it has been shown that covert pathologic change due to lymphatic damage during breast cancer treatment can be detected, and that early intervention in this latent stage can prevent the onset of overt disease [4]. At the low-resourced end of the spectrum, assessment of LF related-lymphedema (LFRL) of the leg usually relies on classification of visible and palpable soft tissue changes [5], where subjectivity may lead to inconsistent classification. There is no differentiation or assessment of covert change, so subtle but important alterations in tissue composition may be missed.

In LF, mosquitoes pick up the microfilariae during a blood meal. The larvae develop to third stage within the mosquito before being transmitted by a subsequent bite. Transmission is relatively inefficient with a low risk of infection per bite, and after transmission there is a lag between being infected and the development of adult worms. This means that most children with LF will remain asymptomatic until young adulthood, which affords a long, latent period in which to implement preventive strategies [6]. Primary prevention in the Global Program to Eliminate LF (GPELF) is preventive chemotherapy, which is delivered annually via mass drug administration (MDA) in endemic regions [7]. This will eventually prevent any new cases of morbidity as infection rates fall too low to sustain transmission. However, preventive chemotherapy conveys no real benefit to advanced cases, most of whom will no longer be antigenemic, but will require life-long health care. In between the asymptomatic cases that will never progress to overt disease and the advanced cases that have irreversible lymphedema, there are many cases of latent and early stage lymphedema. There is some evidence that MDA may reverse very early tissue changes in LFRL [8], but without standardized assessment or diagnostic criteria for Stage 0, or devices sensitive enough to detect small changes in tissue composition, it is not clear at what stage or which individuals will remain at risk of disease progression. Reliable, sensitive, low-cost devices to provide objective assessment of LFRL are needed [9].

A pilot study in Papua New Guinea (PNG) found the skin over the posterior thigh was 20% more compressible in asymptomatic young people who had tested positive for LF antigen compared to antigen-negative peers, using a mechanical tonometer [10]. Subsequently, three tissue tonometers and a portable BIS device have demonstrated intra-operator reliability in assessing tissue composition in the lower limbs of young Australian and Myanmar populations without any history or risk of lymphedema [11]. It is not yet known if covert lymphedema can be detected by tissue tonometry or BIS in these populations.

There is no agreed standard for assessment of Stage 0 lymphedema, and diagnostic criteria for clinical onset are not well defined [3]. One study on BCRL used a 3% change in BIS values to trigger preventive treatment [4], and clinical lymphologists may use a percentage change in limb girth or volume to track lymphedema change, with a variation of more than 10% considered clinically relevant [12]. Variations in body composition will influence measurements with these devices as muscle holds more free fluid than fat, fat is more compressible than muscle, and the ECF in the subcutaneous compartment fluctuates slightly depending on overall body hydration. Individual characteristics that

influence body composition should be considered when assessing superficial tissues of the lower limb, including expected changes associated with growth from child to young adult and gender-based differences in muscle and fat distribution. Habitual patterns of muscle use should also be considered, and significant between-leg differences in healthy young Australian and Myanmar people have been reported when using these devices [13].

This cross-sectional study on young people residing in an LF endemic region in Central Myanmar investigated whether tissue tonometry and BIS measures were altered in asymptomatic cases who tested positive for *Wuchereria bancrofti* antigen. The results will assist researchers and clinicians to objectively quantify changes occurring in early LFRL and may contribute to formal recognition and intervention for Stage 0 lymphedema of the leg.

2. Materials and Methods

2.1. Study Site Selection, Participant Recruitment, and Screening

Sentinel site records kept by the Vector Borne Disease Control (VBDC) Centre in Mandalay identified Amarapura Township as a densely populated area with a high prevalence of LF. It was also close enough to laboratory services for blood sample processing. A study site was set up in the Administration Centre in the village of Nge Toe and baseline data were collected over a two-week period in October 2014. The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Myanmar Ministry of Health (MoH) and James Cook University Human Research Ethics Committee (approval number H5261).

A sample size of 32 in each group was predicted to detect a 10% difference between groups with 80% power, based on a mean mid-calf value of 2.5 with SD of 0.7 using the digital Indurometer [13,14]. A convenience sample of local young people aged 10–21 years was invited to be screened for LF antigen and to participate in a longitudinal study on early detection of LFRL. Participant information sheets and informed consent forms were provided in Burmese. Staff of the VBDC and Amarapura Township Hospital, the World Health Organization (WHO) technical officer for Myanmar (SSW), and locally-trained research assistants explained all procedures to the participants, determined their eligibility to participate, and obtained informed consent. Written consent was given by young adults aged 18–21 and by a parent or guardian for minors aged 10–17. A further verbal assent for each procedure was obtained from all participants prior to performing that procedure. Participant inclusion and exclusion criteria are shown in Figure 1.

Inclusion criteria	Exclusion criteria
• Aged 10 – 21 years at screening • Residing in Amarapura Township • Able to give informed consent (18 – 21 year olds) • Accompanied by an adult relative able to give informed consent (10 – 17 years olds)	• Any clinical sign of lymphedema • Acute injury to the lower limb(s) • Past surgery to the trunk or lower limb(s) • Heart disease • Pacemaker or other implanted device • Pregnancy • Unable to give informed consent

Figure 1. Participant inclusion and exclusion criteria.

2.2. Screening and Baseline Data Collection

A rapid field test for the presence of LF antigen was performed using an immunochromatographic test (ICT) card (Binax Now, Alere, Waltham, MA, USA). This involved placing a 100 µL draw of blood from a fingerprick onto a test strip. The sample was allowed to flow for 10 min and the result appeared as one or two lines across the test strip. One line is a control and if this line was not visible then the test was void and if possible, repeated. Appearance of the second line indicated the presence of circulating *W. bancrofti* antigen that is produced by adult worms. The young people who tested positive by ICT

(cases), and a sample of the negative participants of the same age and gender (controls), were invited to return and participate in the longitudinal study. A James Cook University (JCU) technical staff member (LB) trained the local research assistants in correct use of the ICT card and selected participants invited for follow-up.

Participants returned during the following fortnight for the blood draw and device measures. Local research assistants conducted a short interview to elicit information on current health status, prescription or traditional medications, surgical history, family history of lymphedema, time since the last drink (as a proxy for hydration), and if they had consumed preventive chemotherapy during the previous annual MDA. Leg dominance was determined by asking the question 'Which foot do you use to kick a ball?' Height was measured using a chart marked on a wooden post in centimeters and a set square, and weight in kilograms was recorded using digital scales purchased locally. Device measures were conducted in a small side office or screened off area and an adult relative was asked to be present during the measurement of minors.

2.2.1. Device Measures

Three tissue tonometers were used to assess tissue compressibility. The Indurometer (SA Biomedical Engineering, Adelaide, Australia) is a hand-held electro-mechanical device with a 1 cm diameter plunger/indenter extending through a 7 cm diameter reference plate and a built-in force sensor. The reference plate is aligned to the surface of the skin while the device is pressed evenly into the tissue. A beep is emitted once the equivalent to 200 g of force has been applied, and the degree of displacement is displayed in 0.01 increments on a light-emitting diode (LED) screen. An image of the Indurometer is shown in Figure S1. The mechanical Tonometer (SA Biomedical Engineering, Adelaide, Australia) is a similar device, in which a 1 cm diameter plunger extends beyond a 7 cm diameter reference plate. This purely mechanical device uses a 200 g mass to drive the indenter into the underlying tissue, and the degree of displacement is shown on an analogue scale. Both of these devices record the displacement of the indenter in relation to the reference plate as an indication of compliance (compressibility) of the underlying skin and tissue. The values provided by these devices are not absolute measures and can be considered as arbitrary units used to compare measures of tissue compressibility [15]. A third device, the SkinFibroMeter (Delfin Technologies, Kuopio, Finland), uses a smaller reference plate with a 1.25 mm length fixed indenter and built-in force sensors. The reference plate is pressed evenly onto the skin and the device emits a beep when the equivalent of 50 g has been applied. The device is applied five times and the average resistance in newtons is displayed on a digital screen. A tape measure and washable skin marker were used to locate and mark the midpoint of each thigh (front and back) and the back of each calf, and all tonometry measures were taken at these marks.

Extracellular and intracellular fluid loads were assessed using bio-impedance spectroscopy (BIS), which measures the resistance to multifrequency, low-level electrical currents. The difference between resistance in the intracellular (Ri) and extracellular (Re) fluid compartments was represented as a ratio Ri:Re. As the intracellular fluid (ICF) compartment is tightly regulated, any changes in the ratio usually represent changes in the extracellular fluid (ECF). Whole-leg BIS measures were recorded for each leg with the SFB7 (Impedimed, Australia) using self-adhesive electrodes applied to the skin according to manufacturer's instructions for lower limb measures.

A detailed description of data collection methods was published in a reliability study on these devices in Australia and Myanmar [11]. All devices were operated by the principal researcher (JD), who was blinded to the infection status of the participants. Tonometry scores were recorded on data collection sheets by a research assistant, and BIS data was downloaded to an Excel file (Microsoft Office 365, version 1706).

2.2.2. Blood Collection and Processing/Storage

Blood samples were collected by local research assistants, who were trained in specific blood collection and handling protocols by the JCU technician (LB). A 10 mL draw of venous blood was collected from each participant into cooled ethylenediaminetetraacetic acid (EDTA) anticoagulant vacutainers (BD Biosciences, North Ryde, Australia). The antigen test was repeated using 100 uL of the venous blood pipetted onto an ICT card, and the remaining blood was kept on ice until delivery to the Public Health Laboratory in Mandalay. Separation of plasma and red blood cells was performed using a centrifuge for 15 min at 3000 rpm; the plasma was transferred into 2-mL cryotubes by pipette in duplicate (4 mL per person) and stored at −20 °C. Once all baseline data had been collected, the plasma was transferred on dry ice to the Department of Medical Research in Yangon for long-term storage at −80 °C in a monitored freezer connected to a back-up generator and with daily monitoring. There were no thaws during plasma transportation or storage. One set of the cryotubes was aliquoted and used to conduct ELISA assay for the presence of Og4C3, an antigen marker for *W. bancrofti*, using the recommend 1:4 dilution for plasma as per the manufacturer (Cellabs, Sydney, Australia) kit instructions [16]. Samples were classified as positive if the antigen units, estimated using the standard curve of controls provided with the kit, exceeded 32 units. Detailed methods for the ELISA assays were previously published in a study on diagnostic testing for LF antigen [16].

2.3. Data Analysis

LF antigen-positive cases were defined as those who were positive by either antigen test (ICT or Og4C3). Body mass index (BMI) was calculated as kg/m^2, but adult values cannot be used for children; therefore, WHO growth charts and definitions were used to identify underweight participants, who were defined as being more than two standard deviations below the median BMI for their age [17]. Chi-squared tests, Fisher's exact tests, and independent samples *t*-tests were used to compare antigen-positive and -negative group characteristics at baseline for known moderating factors. Paired sample *t*-tests were used to compare device measures of dominant and non-dominant legs. Statistical analysis was conducted in SPSS version 23 (IBM Corp), and significance was set at 0.05 with a 95% confidence interval. Clinically-relevant difference for tonometry measures was set at >10% and for BIS measures it was set at >3%. Stepwise regression was performed for dominant and non-dominant legs separately to determine the level of variance in device measures associated with infection status (univariate) and other potential moderating factors (multivariate).

3. Results

3.1. Participants

Screening for LF found 60 antigen-positive cases among 316 volunteers, and 114 young people (57 cases and 57 controls of the same age and gender) were invited to continue in the longitudinal study (see Figure 2). Ten people either could not be found or refused to return, and 104 participants were available for baseline blood draw and physical measures. Data from six participants were excluded from the final analysis; four were found at a later measure to have been outside the target age range at baseline, one had a prosthetic leg, and another had a heart condition, neither of which had been disclosed at the screening interview. The final study population was comprised of 46 antigen-positive cases detected by ICT plus a further four cases identified as antigen positive by Og4C3 ELISA (n = 50). There were 48 antigen-negative (control) cases.

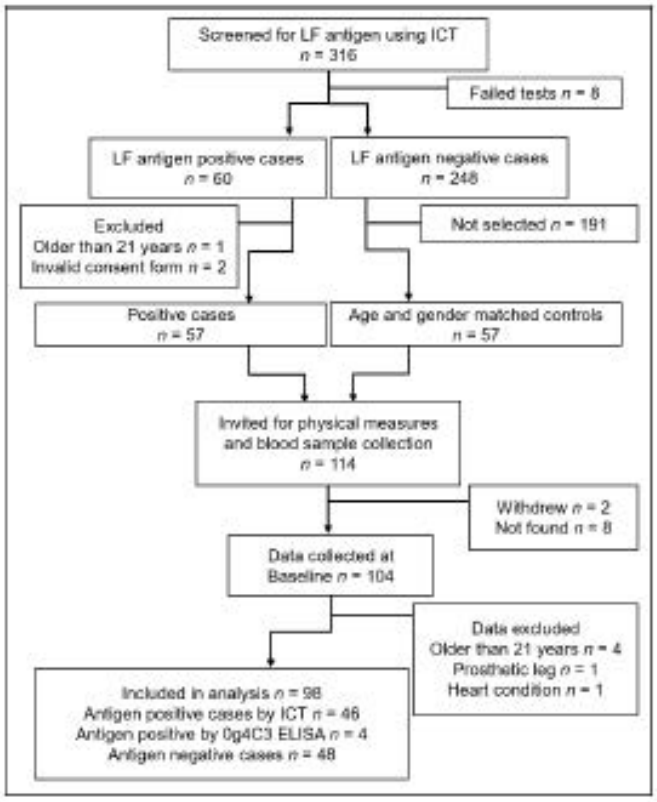

Figure 2. Flow of participants through recruitment, screening, and baseline data collection.

3.1.1. Participant Characteristics

All participants ($n = 98$) were aged between 10 and 21 years (mean 15.3 SD 3.4) and there were 55 females and 43 males. The mean height, weight, and BMI were 152.0 cm (SD 12.0, range 118.8–174.0), 42.3 kg (SD 11.5, range 17.5–82.7), and 18.0 kg/m^2 (SD 3.0, range 12.4–29.7), respectively. The cohort was 95.9% right leg dominant and 13.3% ($n = 13$) were considered underweight. Almost half (44.9%) of the participants were working in weaving workshops, 27.6% were students, 8.2% were street vendors, 2.0% were construction workers, and the remaining 17.3% worked in other occupations or did not disclose their occupation. None had a history of lymphedema in their immediate family, previous surgery or medical implants, and all were in good health. Two participants were taking prescription medications and one was using traditional medicine. One participant felt unwell on the day scheduled for taking the measures and was asked to return when feeling better. Comparing antigen-positive and antigen-negative groups, there were no significant between-group differences for any physical attribute or moderating factor. Participant characteristics at baseline are shown in Table 1.

Table 1. Group characteristics of antigen-positive and antigen-negative participants (positive by either immunochromatographic test (ICT) or Og4C3) at baseline.

	LF Antigen-Positive Cases	LF Antigen-Negative Controls	Mean Diff (95% CI)	*p*=
	n = 50	*n* = 48		
Age in years—mean (SD)	15.20 (3.38)	15.48 (3.46)	0.28 (−1.09, 1.07)	0.691 [a]
Gender				
Female *n* (%)	27 (54%)	28 (58%)		0.410 [b]
Male *n* (%)	23 (46%)	20 (42%)		0.410 [b]
Height in cm—mean (SD)	151.80 (12.56)	152.20 (11.52)	0.399 (−4.44, 5.24)	0.870 [a]
Weight in kg—mean (SD)	42.27 (12.81)	42.30 (10.12)	0.028 (−4.617, 4.670)	0.990 [a]
BMI in kg/m^2—mean (SD)	18.05 (3.46)	18.03 (2.65)	−0.012 (−1.239, 1.216)	0.985 [a]
Body composition *n* = (%)				0.976 [c]
Median weight	41 (82%)	40 (83%)		
Underweight > −2SD	7 (14%)	6 (13%)		
Overweight > +1SD	2 (4%)	2 (4%)		
Dominant leg right/left	47/3	47/1		0.324 [b]
Occupation *n* = (%)				0.395 [c]
Student	14 (28%)	13 (27%)		
Working/other	32/4 (72%)	34/1 (73%)		
Drank liquid *n* = 97				0.590 [c]
<60 min	13 (26%)	12 (26%)		
>60 min	37 (74%)	35 (74%) (1 NA)		
Consumed 2013 MDA *n* (%)	17 (34%)	22 (46%)		0.383 [c]

LF = lymphatic filariasis; BMI = body mass index; SD = standard deviation; [a] independent samples *t*-test; [b] Fishers exact test; [c] Pearson chi-square; NA = participant was not asked.

3.2. Moderating Factors Associated with Device Measures

3.2.1. Effect of Infection on Device Measures

In the antigen-positive group, tissue compressibility was higher at all measuring points, and there was more free fluid in both legs compared to that of the antigen negative group. Independent *t*-tests found that, at mid-calf on the non-dominant side, the increase in Indurometer measures was both clinically (11.1%) and statistically significant ($p = 0.021$). In addition, whole leg BIS measures found clinically-relevant (>3%) increases in free fluid in both legs (dominant leg, 4.9% ($p = 0.220$), non-dominant leg, 9.2% ($p = 0.053$)). Mean values and between-group differences for the Indurometer and BIS measures are shown in Table 2. Neither the mechanical Tonometer nor SkinFibroMeter found any clinically relevant or statistically significant differences between infection groups, with many differences too small to be evident at two decimal places. The only between-group difference of interest with these two devices was increased tissue compressibility with the Tonometer at the non-dominant calf (4.8% softer, $p = 0.296$). Mean values and between-group differences for all devices including the Tonometer and SkinFibroMeter are given in Table S1.

Table 2. Between-infection group differences for Indurometer and BIS measures, size, and direction of variation.

Measurement Point Indurometer	Positive n = 50	Negative n = 48	Mean Difference (%)	Direction in Positive Cases	p=
	Mean (SD)	Mean (SD)			
Dominant anterior thigh	4.80 (0.76)	4.72 (0.69)	0.05 (1.1%)	Softer	0.731
Non-dominant anterior thigh	5.10 (0.88)	5.00 (0.69)	0.10 (1.9%)	Softer	0.546
Dominant posterior thigh	4.13 (0.93)	4.06 (0.87)	0.07 (1.7%)	Softer	0.701
Non-dominant posterior thigh	3.88 (0.83)	3.86 (0.95)	0.02 (0.4%)	Softer	0.933
Dominant calf	2.91 (0.57)	2.70 (0.68)	0.21 (7.8%)	Softer	0.096
Non-dominant calf	2.73 (0.65)	2.46 (0.65)	0.27 (11.1%) *,#	Softer	0.021
BIS					
Dominant leg n = 47/45	2.44 (0.46)	2.56 (0.45)	0.12 (4.9%) #	More fluid	0.220
Non-dominant leg n = 46/44	2.62 (0.56)	2.86 (0.59)	0.24 (9.2%) #	More fluid	0.053

SD = standard deviation; * Significant between-group difference $p \leq 0.05$; # clinically relevant between-group difference.

3.2.2. Effect of Infection Status, Age, Gender, Body Composition, and Hydration on Device Measures

Regression was first performed with infection status (antigen positivity) alone, and then stepwise regression was used to add moderating factors. Being antigen positive was significantly associated with increased compressibility in the non-dominant calf when using the Indurometer (Table 3, step 1) which is consistent with the *t*-test results given above in Table 2. Using multivariate regression, after adjustment for other factors (gender, age, underweight, and hydration), increased compressibility remained significantly associated with being antigen positive (in the non-dominant calf) using the Indurometer, and was also significant in the dominant calf using the same device. When considering all factors, being antigen positive was significantly associated with increased fluid in the non-dominant leg using BIS (Table 3, step 2).

In the stepwise regression, being female was significantly associated with higher tissue compressibility using all three tonometers. The largest gender-related effect using the Indurometer was in calf measures where there is a relatively thin fat layer over the muscles, making small differences in fat and muscle composition more likely to be detected (dominant leg B (SE) = 0.639 (0.117), $p < 0.000$) (see Table 3). The least effect of gender was found over the anterior thighs where the relatively thicker fat layer reduces the influence of the underlying muscle tone and a small difference between the sexes is not likely to register as much change. Using BIS, being female was significantly associated with less free fluid in both legs, and this is consistent with females having relatively smaller muscle/higher fat mass (less fluid) than males of the same age. The largest coefficient was in the non-dominant leg (B (SE) = 0.485 (0.103), $p < 0.001$) (see Table 3).

Being less well hydrated, defined as not having a drink within one hour of measures, was associated with lower tissue compressibility. This was significant at the non-dominant calf (B (SE) = −0.239 (0.110), $p = 0.032$). Being older was significantly associated with a small increase in free fluid in both legs, consistent with normal growth increase in muscle mass. Being underweight was significantly associated with a small increase in free fluid in the non-dominant leg (BIS) which may be associated with reduced fat mass or an increased capillary filtrate due to proteinemia.

In summary, when accounting for known moderating factors of age, gender, BMI, and hydration, there was a highly significant association between antigen positivity and increased Indurometer measures at the non-dominant calf ($p = 0.007$). At the dominant calf, the same association was also significant ($p = 0.038$). When these factors are taken into account for BIS measures, there was a clinically relevant and significant increase in free fluid (Table 2) in the non-dominant leg ($p = 0.038$). All associations between moderating factors and device measures are given in Table S2.

Table 3. Stepwise regression for moderating factors associated with variation in Indurometer and bio-impedance spectroscopy (BIS) measures.

	Indurometer				**BIS**	
	Higher Values = Increased Tissue Compressibility				**Lower Values = Increased ECF**	
	Posterior Thigh B (SE)		Calf B (SE)		Whole Leg B (SE)	
Factor	Dominant	Non−dominant	Dominant	Non−dominant	Dominant	Non−dominant
Step 1 R^2=	*0.002*	*0.000*	*0.029*	*0.054*	*0.017*	*0.042*
Antigen Positive	0.070 (0.182)	0.015 (0.180)	0.212 (0.126)	0.272 (0.116) *	−0.117 (0.095)	−0.238 (0.122)
Step 2 R^2=	*0.189*	*0.187*	*0.283*	*0.269*	*0.283*	*0.398*
Antigen Positive	0.093 (0.168)	0.049 (0.166)	0.234 (0.111) *	0.286 (0.104) **	−0.108 (0.083)	−0.210 (0.099) *
Gender = Female	0.751 (0.178) **	0.679 (0.175) **	0.639 (0.117) **	0.492 (0.110) **	0.230 (0.087) **	0.485 (0.103) **
Older age	0.022 (0.025)	0.041 (0.025)	0.010 (0.017)	0.024 (0.016)	−0.051 (0.012) **	−0.061 (0.015) **
Underweight	0.136 (0.250)	0.277 (0.247)	−0.052 (0.165)	−0.094 (0.155)	−0.237 (0.120)	−0.302 (0.142) *
Less Recent Hydration	−0.338 (0.177)	−0.223 (0.174)	−0.139 (0.117)	−0.239 (0.110) *	0.107 (0.085)	0.124 (0.101)

ECF = extracellular fluid; SE = standard error; * $p < 0.05$; ** $p < 0.01$.

3.3. Patterns of Tissue Compressibility and Free Fluid in Dominant and Non-Dominant Legs

There was a consistent pattern of tissue compressibility at the measurement sites that held true for all devices and all subgroups by age, gender, or infection status. The most compressible

tissue was located at the (relatively) fatty anterior thigh, the least compressible tissue was over the dense tendomuscular junction at mid-calf, and values for the posterior thigh fell between the two. When comparing dominant and non-dominant legs, a consistent pattern of between-leg differences was seen and can be attributed to expected muscle activity during a kick. The skin was less compressible (more muscle tone) over the front of the 'dominant' kicking thigh and over the back of the 'non-dominant' thigh and calf muscles that propel the body forward during the kick. Using BIS, there was more free fluid (more muscle mass or less fat) in the dominant leg compared to the non-dominant leg (9.6%); this difference was both clinically relevant (>3%) and statistically significant ($p < 0.01$) using paired samples *t*-tests. Mean values and between-leg differences for the Indurometer and BIS are given in Table 4. Mean values and between-leg differences for all devices including the Tonometer and SkinFibroMeter and are given in Table S3.

Table 4. Mean values and between-leg differences using the Indurometer and BIS.

	Indurometer (*n* = 98)			**BIS (*n* = 90)**
	Anterior Thigh	**Posterior Thigh**	**Calf**	**Whole Leg**
Dominant leg mean (SD)	4.74 (0.72)	4.10 (0.90)	2.81 (0.63)	2.50 (0.46)
Non−dominant leg mean (SD)	5.05 (0.79)	3.87 (0.89)	2.60 (0.59)	2.74 (0.59)
Mean difference (SD)	−0.31 (0.31)	0.23 (0.23)	0.21 (0.21)	−0.24 (0.32)
95% CI of the difference	−0.41, −0.21	0.11, 0.35	0.13, 0.28	−0.31, −0.17
% difference	6.5% **	5.6% **	7.5% **	9.6% **,#
Direction (dominant leg)	Harder	Softer	Softer	More fluid

SD = standard deviation; ** Significant between-leg difference $p \leq 0.01$; # Clinically relevant between-leg difference (tonometry > 10%, BIS > 3%).

The overall pattern of between-leg differences (dominant vs. non-dominant), as demonstrated by kicking a ball, was maintained in the antigen-positive cases, but the degree of difference was altered. Figure 3 is a radar graph showing the percentage of between-leg differences in Indurometer and BIS values for the whole cohort and by infection group. In the infected group, between-thigh differences in tissue compressibility were exaggerated (closer to the outer ring in the radar chart) but only slightly, with similar percentage differences for positive (7%), negative (6.1%), and whole cohort groups (6.5%). The between-infection group differences were more pronounced at the calf where the mean between-calf difference in the positive cases (6.5%) was much smaller (closer to the middle) than that of the negative cases (9.7%) or whole cohort (7.5%). Similarly, as well as an overall increase in free fluid, BIS results indicated that positive cases had smaller between-leg differences compared to those of their negative counterparts (7.5% vs. 11.7%). Although not statistically significant, these reduced between-leg differences in the distal legs of the antigen-positive cases suggest a covert edema overlying and masking normal between-leg variations in muscle tone and mass.

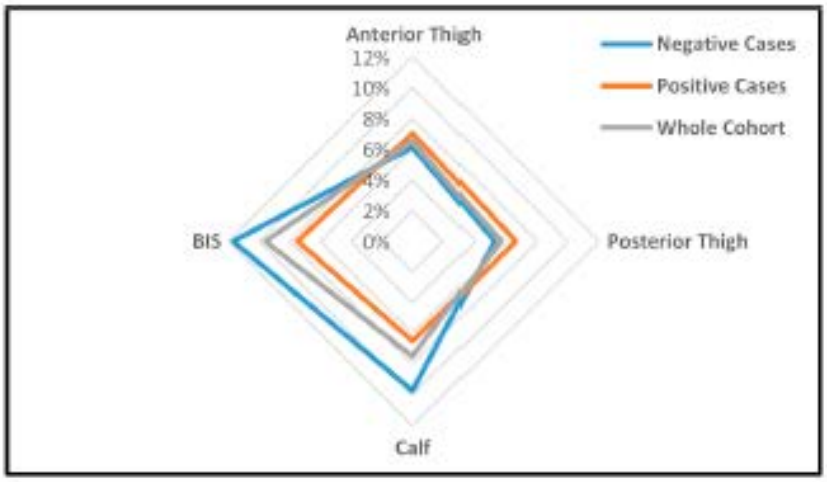

Figure 3. Percentage between-leg differences using the Indurometer and BIS in the LF antigen-negative cases, LF antigen-positive cases, and whole cohort. Data points which are closer to the outer ring indicate greater between-leg differences.

4. Discussion

In this study, tissue compressibility and free fluid loads were higher in asymptomatic young people infected with LF compared to their uninfected peers. Both groups displayed normal patterns of within-leg tissue compressibility; i.e., tissue was most compressible over the anterior thigh and least compressible at the calf, and between-leg differences were consistent with kicking a ball. However, when stratified by infection status, the size and direction of between-leg differences in the positive cases were consistent with a covert accumulation of subcutaneous fluid in the lower leg. Usually, LFRL appears distally and progresses proximally, so detectable tissue changes may occur earlier at the calf than at the thigh. The relatively thin layer of skin and tissue over the muscle of the calf may also render early tissue changes more evident than in fattier parts of the leg. Accordingly, the association with LF antigenemia and Indurometer measures was statistically significant at mid-calf, and large enough on the non-dominant side to also be clinically relevant. This early appearance of lymphatic dysfunction in the non-dominant leg is consistent with reports on BCRL, which show an increased risk of arm lymphedema if the operated side is also the non-dominant arm [18]. This tendency for fluid to accumulate more readily on the non-dominant side could be the result of differences in muscular activity that naturally promotes lymph flow and may be greater or more frequent on the dominant side.

For all devices, the significant associations between higher tissue compressibility and lower free fluid in females reflect expected variation in muscle to fat ratios between the sexes. Other moderating factors such as hydration, although not as universal as gender, did have significant associations with measures at the calf, but this could be reduced by administering a standardized drink during the assessment protocol. Increased free fluid associated with age and being underweight can be attributed to a year-by-year increase in muscle mass, or a systemic reduction in fat mass, respectively.

Results in the Myanmar study reinforce earlier findings from PNG [10], where clinically significant between-infection group differences were found in physical leg measurements. However, some differences in observations between studies were noted. In particular, in young PNG people, increased tissue compressibility was found in the posterior thighs of the infected group using the mechanical Tonometer. In the Myanmar cohort, the between-infection group differences were found using the digital Indurometer at the calf. There may be several reasons for this discrepancy. The PNG cohort had a higher proportion of females (64% vs. 54%) than the Myanmar cohort and a higher mean BMI (19.7 vs. 18.05). In addition, age, gender and hydration were not considered in that analysis. In the current study, the Tonometer did return slightly softer measures in the dominant posterior thigh and non-dominant calf in the Myanmar group, but in this cohort, the differences were not significant. (Table S1). In PNG,

no MDA had been available prior to the study, after which treatment was offered to all participants; in Myanmar, MDA had been offered in 2013 and earlier, although less than half of the participants reported taking it. Taken together, these two studies provide the first empirical evidence that there are covert but measurable increases in tissue compressibility and free fluid associated with LF antigenemia, although the optimal site for assessment may differ for different populations. The advance in the current study over that done in PNG was the availability of newer, digital devices and inclusion of age, gender, BMI, and hydration in multivariate regression, which confirmed an independent effect of infection.

The proportion of all infected individuals that will progress to LFRL, while considered to be relatively small, is not well understood. It appears to depend on multiple factors including genetics, geography, exposure to infection, and worm species, and it was not possible in this cross-sectional study to determine which of the positive cases may be at risk of progression to advanced disease, if any. The fact that mean between-infection group differences can be objectively measured suggests that there is an insidious effect of LF antigenemia on skin and subcutaneous tissues in the lower limb, and this is consistent with the current understanding of the pathogenesis of lymphedema [19,20]. Follow-up on this Myanmar cohort may provide some insight into individual variation among antigen-positive persons to define who is most at risk.

The Indurometer gave the clearest indication that tonometry can be used to detect covert lymphatic change in the lower limb. While the Tonometer and SkinFibroMeter may not have detected latent changes in asymptomatic cases in this cohort, their use in assessment of established leg lymphedema from all causes warrants further study. When using these devices to track changes in the same person over time, moderating factors such as age and gender will be immaterial, hydration can be controlled for by administering a drink prior to measurement, and any change in BMI can be considered when interpreting the results, as is already the practice in BCRL. Indurometry and BIS measures may be useful in monitoring clinical progression in people at risk of lower limb lymphedema and may provide an inexpensive means to objectively measure lymphedema in LF populations.

The presence and direction of clinically-relevant changes in the antigen-positive cases in Myanmar support the hypothesis that LF can induce covert changes in the subcutaneous tissues of the lower limbs. This contributes to the case for formal recognition of a Stage 0 in the classification of LF-related lymphedema. The disparity in resources between BCRL and LFRL settings should not be a barrier to transferring reliable and effective protocols for early detection and intervention in lymphedema to LF populations.

Supplementary Materials: The following are available online at www.mdpi.com/2414-6366/2/4/50/s1, Figure S1: Indurometer, SA Biomedical Engineering; Table S1: Between-infection group differences (independent samples *t*-test) for (a) Digital Indurometer, (b) Mechanical Tonometer, (c) SkinFibroMeter and (d) BIS measures, size and direction of variation; Table S2: Stepwise regression for moderating factors associated with variation in (a) Digital Indurometer, (b) Mechanical Tonometer, (c) SkinFibroMeter and (d) BIS measures; Table S3: Mean values and between-leg differences (paired samples *t*-test) for (a) Digital Indurometer, (b) Mechanical Tonometer, (c) SkinFibroMeter and (d) BIS measures.

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- Impedimed Australia, for loan of an SFB7 back-up unit and donation of electrodes.
- Delfin Finland, for loan of a SkinFibroMeter.
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Conflicts of Interest: The authors declare no conflict of interest.

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Figure S1: Indurometer

Supplementary Material

Table S1: Between-infection group differences (independent samples T-test) for a) Digital Indurometer, b) Mechanical Tonometer, c) SkinFibroMeter and d) BIS measures, size and direction of variation

a) Digital Indurometer (higher values indicate higher tissue compressibility/softer tissue)

Measurement Point	**Positive n=50** Mean (SD)	**Negative n=48** Mean (SD)	**Mean (%) difference**	**Positive cases are**	**p=**
Dominant Anterior Thigh	4.77 (0.76)	4.72 (0.69)	-0.05 (1.1%)	Softer	0.731
Non-dominant Anterior Thigh	5.10 (0.88)	5.00 (0.69)	-0.10 (1.9%)	Softer	0.546
Dominant Posterior Thigh	4.13 (0.93)	4.06 (0.87)	-0.07 (1.7%)	Softer	0.701
Non-dominant Posterior Thigh	3.88 (0.83)	3.86 (0.95)	-0.02 (0.4%)	Softer	0.933
Dominant Calf	2.91 (0.57)	2.70 (0.68)	-0.21 (7.8%)	Softer	0.096
Non-dominant Calf	2.73 (0.65)	2.46 (0.65)	-0.27 (11.1%)*#	Softer	0.021

b) Mechanical Tonometer (higher values indicate higher tissue compressibility/softer tissue)

Measurement Point	**Positive n=50** Mean (SD)	**Negative n=48** Mean (SD)	**Mean (%) difference**	**Positive cases are**	**p=**
Dominant Anterior Thigh	6.55 (0.92)	6.55 (0.93)	0.00^	Equivocal	0.998
Non-dominant Anterior Thigh	7.04 (1.09)	7.09 (0.86)	0.05 (0.7%)	Equivocal	0.815
Dominant Posterior Thigh	5.65 (1.16)	5.57 (1.20)	-0.08 (1.3%)	Softer	0.753
Non-dominant Posterior Thigh	5.47 (0.95)	5.57 (1.02)	0.10 (1.8%)	Harder	0.629
Dominant Calf	4.29 (0.85)	4.23 (0.98)	-0.06 (0.3%)	Equivocal	0.766
Non-dominant Calf	4.18 (0.86)	3.99 (0.88)	-0.19 (4.8%)	Softer	0.296

^ absolute differences were at the 3rd decimal place therefore meaningful % could not be calculated.
* Significant between-group difference p≤0.05
** Significant between-group difference p≤0.01
# Clinically relevant between-group difference (tonometry >10%, BIS >3%)

Cont….

c) SkinFibroMeter (lower values indicate higher tissue compressibility/softer tissue)

Measurement Point	**Positive n=50 Mean N (SD)**	**Negative n=48** Mean N (SD)	**Mean (%) difference**	**Positive cases are**	**p=**
Dominant Anterior Thigh	0.08 (0.02)	0.07 (0.01)	0.00^	Equivocal	0.386
Non-dominant Anterior Thigh	0.07 (0.01)	0.07 (0.01)	0.00^	Equivocal	0.890
Dominant Posterior Thigh	0.08 (0.02)	0.08 (0.02)	0.00^	Equivocal	0.917
Non-dominant Posterior Thigh	0.09 (0.02)	0.08 (0.02)	0.01^	Harder	0.251
Dominant Calf	0.10 (0.04)	0.10 (0.03)	0.00^	Equivocal	0.524
Non-dominant Calf	0.11 (0.03)	0.11 (0.03)	0.00^	Equivocal	0.974

d) Bio-impedance spectroscopy (Ri:Re, lower values indicate more free fluid)

Measurement Point	**Positive n=50 Mean Ri:Re (SD)**	**Negative n=48** Mean Ri:Re (SD	**% difference**	**Positive cases are**	**p=**
Dominant Leg n=47/45	2.44 (0.46)	2.56 (0.45)	0.12 (4.9%)#	More fluid	0.220
Non-dominant Leg n=46/44	2.62 (0.56)	2.86 (0.59)	0.24 (9.2%)#	More fluid	0.053

^ absolute differences were at the 3rd decimal place therefore meaningful % could not be calculated.
* Significant between-group difference p≤0.05
** Significant between-group difference p≤0.01
# Clinically relevant between-group difference (tonometry >10%, BIS >3%)

Table S2: Stepwise regression for moderating factors associated with variation in a) Digital Indurometer, b) Mechanical Tonometer, c) SkinFibroMeter and d) BIS measures

a) Digital Indurometer (higher values indicate higher tissue compressibility)

		Anterior Thigh *B (SE)*		**Posterior Thigh *B (SE)***		**Calf *B (SE)***	
	Factor	**Dominant**	**Non-dominant**	**Dominant**	**Non-dominant**	**Dominant**	**Non-dominant**
Step 1	R^2 =	*0.001*	*0.004*	*0.002*	*0.000*	*0.029*	*0.054*
	Antigen Positive	0.0514 (0.147)	0.097 (0.160)	0.070 (0.182)	0.015 (0.180)	0.212 (0.126)	0.272 (0.116)*
Step 2	R^2 =	*0.066*	*0.044*	*0.189*	*0.187*	*0.283*	*0.269*
	Antigen Positive	0.060 (0.146)	0.104 (0.161)	0.093 (0.168)[1]	0.049 (0.166)	0.234 (0.111)*	0.286 (0.104)**
	Gender = Female	0.275 (0.154)	0.330 (0.170)	0.751 (0.178)**	0.679 (0.175)**	0.639 (0.117)**	0.492 (0.110)**
	Older age	0.026 (0.022)	-0.012 (0.024)	0.022 (0.025)	0.041 (0.025)	0.010 (0.017)	0.024 (0.016)
	Under-weight	0.079 (0.216)	0.085 (0.239)	0.136 (0.250)	0.277 (0.247)	-0.052 (0.165)	-0.094 (0.155)
	Less recent Hydration	-0.209 (0.153)	-0.095 (0.169)	-0.338 (0.177)[2]	-0.223 (0.174)	-0.139 (0.117)	-0.239 (0.110)*

b) Mechanical Tonometer (higher values indicate higher tissue compressibility)

		Anterior Thigh *B (SE)*		**Posterior Thigh *B (SE)***		**Calf *B (SE)***	
	Factor	**Dominant**	**Non-dominant**	**Dominant**	**Non-dominant**	**Dominant**	**Non-dominant**
Step 1	R^2 =	*0.000*	*0.001*	*0.001*	*0.002*	*0.001*	*0.011*
	Antigen Positive	0.000 (0.187)	-0.046 (0.798)	0.075 (0.239)	-0.097 (0.199)	0.055 (0.185)	0.185 (0.176)
Step 2	R^2 =	*0.165*	*0.122*	*0.272*	*0.226*	*0.366*	*0.309*
	Antigen Positive	0.029 (0.175)	-0.016 (0.190)	0.106 (0.209)	-0.064 (0.180)	0.093 (0.151)	0.218 (0.151)
	Gender = Female	0.468 (0.185)*	0.682 (0.201)**	1.062 (0.221)**	0.866 (0.190)**	1.122 (0.160)**	0.923 (0.159)**
	Older age	0.071 (0.026)	0.008 (0.029)	0.043 (0.031)	0.036 (0.027)	0.014 (0023)	0.030 (0.023)
	Under-weight	0.268 (0.260)	0.265 (0.283)	0.554 (0.311)	0.267 (0.267)	0.206 (0. 225)	0.180 (0.224)
	Less recent Hydration	-0.292 (0.184)	-0.097 (0.200)	-0.689 (0.220)**	-0.376 (0.189)*	-0.341 (0. 159)*	-0.342 (0.158)*

* $p \leq 0.05$
** $p \leq 0.01$

Contd….

c) SkinFibroMeter (lower values indicate higher tissue compressibility)

		Anterior Thigh B (SE)		Posterior Thigh B (SE)		Calf B (SE)	
	Factor	**Dominant**	**Non-dominant**	**Dominant**	**Non-dominant**	**Dominant**	**Non-dominant**
Step 1	R^2 =	*0.008*	*0.000*	*0.000*	*0.014*	*0.004*	*0.000*
	Antigen Positive	0.003 (0.003)	0.000 (0.003)	0.000 (0.004)	0.006 (0.005)	0.005 (0.007)	0.000 (0.006)
Step 2	R^2 =	*0.238*	*0.192*	*0.263*	*0.191*	*0.226*	*0.153*
	Antigen Positive	0.002 (0.003)	0.000 (0.002)	-9.027E-5 (0.004)	0.005 (0.005)	0.004 (0.007)	-0.001 (0.006)
	Gender = Female	-0.014 (0.003)**	-0.012 (0.003)**	-.022 (0.004	-0.021 (0.005)**	-0.033 (0.007)	-0.022 (0.006)
	Older age	-0.001 (0.000)	-2.585E-5 (0.000)	.000 (0.001	0.000 (0.001)	0.000 (0.001)	-0.001 (0.001)
	Under-weight	0.001 (0.004)	-0.002 (0.004)	.004 (0.006	0.006 (0.007)	0.002 (0.010)	0.009 (0.009)
	Less recent Hydration	0.007 (0.003)*	0.002 (0.003)	.008 (0.004	0.004 (0.005)	0.019 (0.007)	0.013 (0.006)

d) Bio-impedance spectroscopy (SBF7) (lower values indicate higher free fluid)

		Whole Leg B (SE)	
	Factor	**Dominant Leg**	**Non-dominant Leg**
Step 1	R^2 =	*0.017*	*0.042*
	Antigen Positive	-0.117 (0.095)	-0.238 (0.122)
Step 2	R^2 =	*0.283*	*0.398*
	Antigen Positive	-0.108 (0.083)	-0.210 (0.099)*
	Gender = Female	0.230 (0.087)**	0.485 (0.103)**
	Older age	-0.051 (0.012)**	-0.061 (0.015)**
	Under-weight	-0.237 (0.120)	-0.302 (0.142)*
	Less recent Hydration	0.107 (0.085)	0.124 (0.101)

* $p \leq 0.05$
** $p \leq 0.01$

Table S3: Mean values and between-leg differences (paired samples t-test) for a) Digital Indurometer, b) Mechanical Tonometer, c) SkinFibroMeter and d) BIS measures

a) Digital Indurometer n=98 (higher values indicate higher tissue compressibility)

	Dominant leg Mean (SD)	Non-dominant leg Mean (SD)	Mean difference (SD)	95% CI of the difference	% difference	Direction of variation Dominant leg	p=
Anterior Thigh	4.74 (0.72)	5.05 (0.79)	-0.31 (0.31)	-0.41, -0.21	6.5%**	Harder	p<0.001
Posterior Thigh	4.10 (0.90)	3.87 (0.89)	0.23 (0.23)	0.11, 0.35	5.9%**	Softer	p<0.001
Calf	2.81 (0.63)	2.60 (0.59)	0.21 (0.21)	0.13, 0.28	7.9%**	Softer	p<0.001

b) Mechanical Tonometer n=98 (higher values indicate higher tissue compressibility)

	Dominant leg Mean (SD)	Non-dominant leg Mean (SD)	Mean difference (SD)	95% CI of the difference	% difference	Direction of variation Dominant leg	p=
Anterior Thigh	6.55 (0.92)	7.06 (0.98)	-0.52 (0.76)	-0.67, -0.36	7.9%**	Harder	p<0.001
Posterior Thigh	5.61 (1.18)	5.52 (0.98)	0.09 (0.80)	-0.07, 0.25	1.6%	Softer	0.273
Calf	4.26 (0.91)	4.09 (0.87)	0.18 (0.56)	0.06, 0.29	4.1%**	Softer	0.002

c) SkinFibroMeter n=98 (lower values indicate higher tissue compressibility)

	Dominant leg Mean (SD)	Non-dominant leg Mean (SD)	Mean difference (SD)	95% CI of the difference	% difference	Direction of variation Dominant leg	p=
Anterior Thigh	0.07 (0.02)	0.07 (0.01)	0.01 (0.01)	0.00, 0.01	8.8%**	Harder	p<0.001
Posterior Thigh	0.08 (0.02)	0.09 (0.02)	-0.00 (0.02)	-0.01, 0.00	1.9%	Softer	0.424
Calf	0.10 (0.04)	0.11 (0.03)	-0.01 (0.03)	-0.01, -0.00	7.2%*	Softer	0.014

d) Bio-impedance spectroscopy (SBF7) n=90 (lower values indicate higher free fluid)

	Dominant leg Mean (SD)	Non-dominant leg Mean (SD)	Mean difference (SD)	95% CI of the difference	% difference	Direction of variation Dominant leg	p=
Whole Leg	2.50 (0.46)	2.74 (0.59)	-0.24 (-0.32)	-0.31, -0.17	9.6%**#	More fluid	p<0.001

* Significant between-leg difference p≤0.05
** Significant between-leg difference p≤0.01
# Clinically relevant between-leg difference (tonometry >10%, BIS >3%)

Appendix F5: List of oral presentations

2015 American Society of Tropical Medicine and Hygiene, 64th Annual meeting, Philadelphia USA.

Detecting sub-clinical change in young people at risk of lymphatic filariasis related lymphoedema

2016 Australia Pacific Lymphology Conference, Darwin Australia

Reliability of 3 tonometry devices and BIS in the legs of healthy young people

DOI: 10.13140/RG.2.2.31889.22887

2016 Australia Pacific Lymphology Conference, Darwin Australia

Standing on One Leg. LF Elimination by 2020??

DOI: 10.13140/RG.2.2.15112.01285

2016 44th Myanmar Health Research Congress, Yangon Myanmar.

Early Detection of Lymphatic Disturbance in Adolescents Infected with Lymphatic Filariasis

2016 Liverpool School of Tropical Medicine, LSTM Seminar Series, Liverpool UK.

Lymphatic Filariasis in Myanmar, Morbidity, MDA and Detection of Early Lymphatic Disease

DOI: 10.13140/RG.2.2.18467.45605

Appendix G: Permission to use photographic images

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