A comparison of three intervention methods for women with breast cancer-related lymphedema: a pilot study

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ABSTRACT

The incidence of breast cancer related lymphedema (BCRL) is estimated to be at 30% for women who undergo breast cancer treatment, thus creating the need for effective treatment interventions. This thesis will compare the immediate and short-term effects of three different interventions for BCRL (e.g., Manual Lymphatic Drainage (MLD), Aqua Lymphatic Therapy (ALT), and a Casley-Smith based exercise routine (CSER)) and to record any changes in physical symptoms after each intervention. Sixteen women between the ages of 35 and 75 with Stage II lymphedema were recruited. Each intervention was completed once by each participant and lasted approximately 45 minutes. Bilateral limb measurements to estimate changes in volume were performed using bio-impedance spectroscopy and circumferential arm measures. The Lymphedema Breast Cancer Questionnaire was utilized to subjectively gauge any change in symptoms. The main findings were: ALT was the only intervention that showed a significant ($p \leq 0.05$) decrease in extracellular fluid (ECF) from baseline to 20-24hrs post-intervention. When compared to the MLD, the ALT intervention demonstrated a 3.31% greater ($p=0.038$) reduction in ECF volume over 24hrs. With respect to circumferential arm measurements, there was a significant difference ($p=0.021$) in the percentage change between ALT and MLD at 10 min post intervention. No other circumferential arm measurement differences exist between ALT vs CSER or MLD vs CSER. There were no changes or any worsening symptoms following any interventions. Conclusion: In the immediate to short-term post-intervention period, ALT appears to be the most effective intervention in terms of reducing BCRL.

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Contributions

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Table 1: Abbreviations

ALT	Aqua Lymphatic Therapy
BCRL	breast cancer related lymphedema
BIS	bioelectric impedance spectroscopy
CB	multi-layered compression bandaging
CDT	Complete (or Complex) Decongestive Therapy combination of MLD, compressive garment, exercise instruction and skin care
CM	circumferential arm measurements with tape measure
CDT	Complete (or Complex) Decongestive Therapy
CS	compressive sleeve
CSER	Casley-Smith based exercise routine
ECF	extracellular fluid
HR	Hazard ratio: LE edema volume increase of ≥ 50%
LBCQ	Lymphedema Breast Cancer Questionnaire
SLD	Simple lymphatic drainage (self-massage)
SM	Self-management
SP	Standard physiotherapy: bandage, elevation, head–neck, shoulder exercises and skin care
TCvol:	truncated cone volume calculation

INTRODUCTION

The primary focus of this thesis is centered on the assessment and treatment of breast cancer related lymphedema (BCRL). The project was coordinated and all outcome measures were performed by the author at the McGill Nutrition and Performance Laboratory of the McGill University Health Center. This thesis contains the following two manuscripts: 1) "*Determining the precision of dual energy x-ray absorptiometry and bioelectric impedance spectroscopy in the assessment of breast cancer-related lymphedema* (accepted for publication by Lymphatic Research and Biology) and 2) ("A c*omparison of three intervention methods for women with breast cancer-related lymphedema: a pilot study*").

The incidence of breast cancer-related lymphedema (BCRL) is approximately 30% for women who undergo breast cancer treatment (e.g. mastectomy, axillary node dissection and/or radiation treatment) (Williams A, 2005), (Petrek, Senie, Peters, & Rosen, 2001). BCRL is a condition that results in swelling of the arm(s), hand(s) and/or breast(s) initially caused by an accumulation of protein-rich interstitial fluid. The composition of BCRL has been shown to evolve from the initial accumulation of fluid to the development of more solid depositions such as fibrotic lesions and abnormal fat accumulation.

Therefore, since the development of BCRL appears to evolve from a fluid to a more solid consistency, precise and reliable measures from an assortment of different assessment tools are required to develop accurate staging and management. Techniques such as dual-energy x-ray absorptiometry (DXA) and bioelectric impedance spectroscopy (BIS) are two measurement devices that are gaining prominence and acceptance in the lymphedema research community with regards to their safety, ease of measurement and ability to estimate soft tissue and fluid components, respectively (Gjorup C, 2010), (Czerniec S. A, 2010),(Paskett E.D, 2012). There

is little published information on the precision of these two instruments for edematous upper extremities, particularly when they are both derived from one cohort of women on the same day. This is the topic of the first manuscript within this thesis.

Velanovich (1999) revealed that for a significant number of patients with BCRL the disorder is physically as well as emotionally disabling. The physical symptoms (e.g., stiffness, numbness, and fatigue) and functional outcomes (e.g., decreased strength and range of motion) place a constant and significant burden on women with BCRL that affects their quality of life (Passik, 1998). Although there are a growing number of different treatment interventions, little is known about the efficacy of these treatments in terms of either maintaining or reducing BCRL. The second study with two measures of limb volume as outcome measures, investigated the following three treatment interventions: 1) ALT, 2) MLD, and 3) CSER) over a 24 hour period. As an introduction to the topic the following review of literature addresses what is known about the three treatment interventions and detailed chapters covering all aspects of the research undertaken.

CHAPTER 1: REVIEW OF THE LITERATURE

This literature review contains an overview of BCRL and an examination of three modalities (e.g., Manual Lymphatic Drainage, Aqua Lymphatic Therapy, and Casley-Smith based exercise routine) that are used to maintain or reduce limb volume associated with BCRL. Specifically, studies will be reviewed as a means of examining how effective these modalities are for limiting the progression of BCRL.

Background

BCRL is a chronic condition that results in swelling of the arm(s), hand(s) breast, and /or chest wall caused by an accumulation of protein-rich interstitial fluid. This increase in fluid is generally the result of surgical interventions and medical treatment for breast cancer (e.g. mastectomy, lumpectomy, axillary node dissection and radiation) that has damaged the lymphatic vessels of the axillary region inhibiting the clearing of intercellular fluid by the lymphatic system to the venous system (Thomas-MacLean R, 2008). Lymph fluid consists primarily of proteins, water, fatty acids, salts, white blood cells (e.g. T-cells, macrophages) and microorganisms. As a result of the obstructions or the removal of the primary lymphatic channels, the stagnant lymph significantly changes the dynamics of capillary fluid exchange such that interstitial colloid osmotic pressure increases creating localized edema. The subsequent swelling interferes with the oxygenation of the tissues ($\downarrow PO_2$ and $\uparrow PCO_2$) in the surrounding area reducing healing and function (Matheus, 2011).

Table 2: Stages of lymphedema according to International Society of Lymphology

Stage	Description of the Stages of Lymphedema	
0	Subclinical state where swelling is not evident despite impaired lymph transport. This stage may exist for months or years before edema becomes evident	
I	This represents early onset of the condition where there is accumulation of tissue fluid that subsides with limb elevation. The edema may be pitting* at this stage	
II	Limb elevation alone rarely reduces swelling and pitting is manifest	Late stage II: There may or may not be pitting as tissue fibrosis is more evident
III	The tissue is hard (fibrotic) and pitting is absent. Skin changes such as thickening, hyper pigmentation, increased skin folds, fat deposits and warty overgrowths develop	

*Pitting: an indentation made with a finger that persists for some time after the release of pressure

Progression of this chronic condition (e.g. lymphatic stasis and protein-rich edema) will eventually lead to functional and structural changes within the dermis and subcutaneous tissue as evidenced by the development of fibrotic lesions and abnormal fat deposition (Tassenoy A, 2009). There is strong agreement among the experts in the field that in most cases BCRL is a chronic, incurable condition that will gradually progress without treatment (Cornish, et al., 2001), (Bar Ad, et al., 2010), (Piller & Carati, 2009). through a number of different stages. The progression of lymphedema is classified into four stages according to the International Society of Lymphology (Lymphology, 2009) (see Table 2).

Along with hand, arm and breast swelling, other physical symptoms of BCRL include arm tenderness**,** soreness, numbness**,** aching, pain**,** heaviness, fatigue**,** tightness, firmness, stiffness and recurrent infections as well as psychological, social, sexual, and functional problems (Fu, 2009). The appropriate treatment upon diagnosis of BCRL, as outlined in the Position Statement of the National Lymphedema Network, is Complex (or complete or combined) Decongestive Therapy (CDT), also called Complex Physical Therapy CDT consists of the following two phases: Phase I (limb volume reduction) is an intensive 2-6 week course of treatment with a lymphedema specialist that includes 4 components: 1) MLD; 2) skin care; 3) daily compression bandaging used to achieve a compression gradient and; 4) remedial exercises (Network, 2009). The two main goals of this phase are to reduce the volume of the lymphademetous limb and to attend to any skin irritations and to prevent infections that are common sequelae to a condition of impaired lymphatic function. Phase I continues until the limb volume reduction has reached a plateau for 2-3 days at which point the patient moves into the next phase. Phase II of the CDT protocol is considered to be a maintenance period. For the most part, the patients themselves are responsible for Phase II and will need to take self-directed measures for the rest of their lives to maintain the limb volume reduction. Standard practice for clinicians treating BCRL is to educate the patient throughout Phase I on skin care and refer to lymphedema therapists and remedial exercise instructors for instruction on self- massage and home-based exercises (Lawenda, Mondry, & Johnstone, 2009). This is to facilitate the self management required in Phase II for the maintenance of the limb volume reduction. At the end of Phase I, the clinician fits the patient with a compressive garment to be worn on the arm according to need. For some, the garment needs to be worn throughout the waking hours but, others may have to wear the compressive garments over the entire day and night for the long term maintenance of

their arm volume. The other components of Phase II are: 1) continued skin care; 2) remedial exercises; 3) compressive bandaging; 4) MLD and; 5) self massage.

The three treatment interventions (MLD, ALT and CSER)

The focus of this review is MLD and two different remedial exercise routines (ALT and CSER) and their effectiveness in reducing and or maintaining the limb volume in BCRL. In 1936, Emil Vodder (Williams A. , 2010) developed and was the first to present MLD as a method of massage for lymphatic drainage. This method of massage incorporates gentle circular movements in specific directions, using an approximate pressure of 30mmHg, and rest periods stretching the skin and attempting to encourage lymph flow via lymphatic contractions. The CSER was specifically created for the treatment of upper and lower limb lymphedema (Casley-Smith J.R, 1998). This exercise routine involves daily low-intensity exercises of 20-45 minutes performed in a particular sequence to theoretically augment the normal lymphatic pumping action of muscles (Havas E P. T., 1997). ALT is a water-based intervention that takes advantage of the natural hydrostatic pressure gradient of the swimming pool. The exercises involve the shoulder girdle, abdomen, head and neck, and then the distal arm. In addition to causing a massage effect, the limb position and direction of movement during exercise in the water encourage lymph flow away from the affected arm.

Methods

Search strategy: A survey of the literature was carried out using the electronic database PubMed/ (Medline) entering the following keywords and phrases: manual lymphatic drainage; Casley-Smith exercise or aqua-therapy or immediate treatment, and breast cancer related lymphedema (BCRL); breast cancer and arm swelling; BCRL and maintenance. All titles were screened and abstracts of potentially relevant articles were analyzed. Criteria for inclusion in

this review were fourfold: 1) BCRL studies published within the last ten years; 2) being available in English through open access; 3) MLD, ALT or Casley-Smith based exercise (CSER) as one level of the independent variable and; 4) limb volume as one of the outcome measures.

Review of Literature

Though there have been no randomized clinical trials measuring the efficacy of CSER as the sole intervention, there was one cross-sectional study group report of eight women attending 8 x-1 hr classes per week of CSER (Bracha, 2010). The outcome measures of this study were pre- and post- limb volume and Quality of Life. The results showed that the average reduction of limb volume after each class was 26ml with a range of 12-44ml. After eight weeks, 5 women had achieved volume reduction >15% with a range of 16-88%. The remaining three women had maintained their pre-intervention arm volume measurements.

With respect to ALT, four research articles were found; however, only one article satisfied the selection criteria. In their single-blind, randomized clinical trial, Tidhar & Katz-Leurer (2010) recorded the long-term effects of ALT. The treatment group participated in weekly ALT classes for 3 months and the control group was asked to perform self management and was given exercise and self-massage instruction booklets. The major findings included a significant and immediate post intervention reduction in limb volume; however, no significant volume reduction was observed at the end of the 3-month study period. Of interest, the adherence rate for the ALT classes (79%) was significantly greater than the self-management (<30%) over the same period.

The body of knowledge on the two exercise interventions (e.g. ALT & CSER), is greatly lacking with only one recent publication for each (Bracha, 2010), (Tidhar D., 2010).

For MLD, seventeen research articles were found of which 7 studies were excluded because MLD was utilized for cellulite reduction (1); limb volume was not an outcome measure (2); studies assessing leg lymphedema (2) or non cancer treatment related limb edema (2). Ten studies investigating the effectiveness of MLD are presented in Table 3. In total, including the the studies from the other two interventions, this review includes 12 research studies with a total of 1592 participants.

The comparison of the 10 MLD studies in this review is challenging due to the heterogeneity of the study designs. For example, the duration of interventions varies from one treatment session to 4 years of treatment. The comparative variables vary from counseling to self-massage to CDT. A similarity amongst the studies was that 8 studies utilised circumferential measures of limb volume as an outcome measure.

This review identifies five studies that compare MLD with CDT (e.g. compressive bandaging, compressive sleeve, remedial exercise and skin care), or one or more of the CDT components. The results from these studies are mixed. Starting with the studies that address measurements after a short-term course of MLD treatment, Maher et al. (2012) combined MLD with the use of the compressive sleeve and compared it with MLD alone in a single session design using BIS and perometry to measure any volume changes at 30min intervals for two hours. No significant change in volume for either intervention was observed. The sample size in this study was small with n=15 in each group. Another weakness was having the assessments done by the MLD therapist that may have lead to a measurement bias.

A randomized control trial compared standard physiotherapy (SP: bandage, elevation, head–neck, shoulder exercises and skin care) group (n=26) to CDT group including MLD (n=27) all received treatment three times per week for four weeks (Didem, 2005). The findings were

significant with a mean volume reduction for both the SP group (36%) and the CDT group (55.7%). When comparing these two groups the volume reduction was significantly greater for the CDT group ($p< 0.005$).

McNeely (2004) compared MLD & CB group (n=24) to CB group (n=21) over a 4-week period where the MLD group received 5 treatments per week . Significant volume reductions in the both groups were observed and the authors concluded that there is no significant difference in volume reduction with or without the use of MLD.

Four of the ten MLD related studies were carried out over a period of 12-months or longer. Koul et al. (2007) assigned one group (n=76) a combined MLD & CDT intervention and compared it with a group (n=44) receiving MLD alone over a 12-month period. Their findings showed MLD alone reduced limb volume significantly yet; the reduction was 10% higher when CB and exercises were also part of the treatment. There were several limitations to this study including the fact that the participants were not randomized to the different groups and the treatment frequency was left up to the discretion of the individual therapists.

Vignes et al., (2006; 2010) have reported on two, long-term prospective studies. Their initial study compared three components (MLD, CB, and CS) following two 6-month intervals of the Phase II maintenance program in 358 BCRL patients (Vignes S, 2006). Significant increases in limb volume were found with CB ($p<0.0001$) and CS ($p=0.002$) but not MLD and it was concluded that noncompliance was the key risk factor in determining this outcome after one year. Some of the limitations of this study include non-randomization of participants to the treatments, lack of a systematic method of tracking compliance to the treatments and the authors indicate a high proportion of participants were lost to the 12-month follow-up. The second and longer prospective study was conducted over a four year period with milestone

comparisons at 1, 2, and 4 years. A total of 682 patients were assessed and evaluated with MLD, CS and the combination of interventions (e.g., MLD & CB and MLD&CS) (Vignes S., 2009). The measurements at 12 months were used to calculate the "Hazard Ratio" (HR) (1.00 being the ratio that identified a limb volume increase > 50% above baseline). The lowest HR was found with the group using CB & CS with a ratio number less than 1.00 (p=0.004); MLD (HR > than 1.00 p=0.03) alone was the worst. The higher ratio indicates a higher likelihood of increasing limb volume > 50 %. These results indicate that in the long term a maintenance program containing MLD alone is not of benefit to these women with BCRL. The study weaknesses include 1) non-randomizing of patients to treatment and 2) the self-recording of compliance to treatments which may contain some patient error.

Reul-Hirche (2011) carried out a randomized controlled trial over a 12-month period with 160 breast cancer survivors having undergone axillary lymph node dissection. The outcome measure was the incidence of LE. The patients were randomized to one of two groups. The first group (n=81) received guidelines for LE prevention and exercise training. The second group (n=79) received the same guidelines and exercise training and 40 sessions of MLD. The authors concluded that there was no additional benefit of MLD since there were no differences in the incidence of LE at 3, 6, or 12 months. Furthermore, the addition of MLD to guidelines for LE prevention and exercise training does not appear to prevent the onset of LE in the 1st year after surgery for axillary lymph node dissection.

Three of the ten studies involved Simple Lymphatic Drainage (SLD), which is a layman's variation on MLD involving the same sequence of movements and massage done by the patients on themselves following the individualized instruction by a lymphedema therapist. Two of the studies compared this form of self-massage (SLD) with MLD. In the first of these studies,

Williams et al. (2002) using a cross-over design for a 21-day intervention treatment period in 29 patients, found a significant mean limb volume decrease of 71ml (p=0. 013) with the MLD with small (30mls) insignificant changes with SLD. With similar results, the second study involving MLD and SLD compared the percentage change in excess limb volume over a 14-day treatment period and found a significant decrease of 33.8% with MLD (n=15), but no significant change with SLD (n=13) (Sitzia, Sobrido, & Harlow, 2002). However, neither of these studies found a significant difference between SLD and MLD in terms of the decreases in limb volume. Taking into consideration the relatively small sample sizes of these two studies, it would be premature to state that MLD is relatively more effective than SLD.

Finally, a crossover study that compares self-massage with a garment, which utilizes a compression pump, that is purported to simulate the actions of MLD was added to the review for its novel perspective and its contribution to the body of knowledge on self-massage (Wilburn O, 2006). This crossover study by Wilburn et al. (2006) involved a small group (n=10) participating who were randomly assigned into two, 14-day treatment periods; one with SLD and the other with the Flexitouch™ (mechanical pressure garment). The results of this research demonstrated a significant mean volume reduction of 208ml ±157ml (p=0.002) over time with the Flexitouch™ and a non-significant mean volume increase of 52ml ± 106ml with SLD. The post-treatment volume differences between the two interventions found the Flexitouch™ garment had a significantly greater decrease (p=0.007) in volume compared to the SLD. Although the evidence is compelling to select this mechanical device over SLD, from a pragmatic perspective, this device is neither easily available nor affordable (>$10,000) to the majority of patients living with BCRL.

Table 3: Selected studies describing the treatment efficacy for women with BCRL

Author	Sample size / Design	Treatment	Volume Measurements	Results
Studies involving CSER & ALT				
Bracha et al. (2010)	n=8 Case report	8wk once/week 1hr CSER	Bilateral limb TCvol, CM, from the wrist to axilla at 5-cm intervals, QoL	Only : significant* improvement Emotional & Social QoL
Tidhar et al (2010)	n=48 Single-blind randomized clinical trial	ALT + SM n=16 SM (Control) n=32 3mos Tx	Water displacement Adherence diary QoL	ALT: Significant* immediate vol ↓ ; no significant volume reduction at 3mos ALT: significantly*greater adherence rate than SM
Studies involving MLD				
Didem et al. (2005)	n=53 randomized clinical trial	CDT n = 27 SP n =26 3x/wk for 4wks	Bilateral limb water displacement volumetry & TCvol CM from the wrist to axilla at 5-cm intervals, ROM	CDT significantly*greater vol ↓ than SP Both Significant* ↑ ROM
Koul et al. (2007)	n= 138 Retrospective	CDT n=76 MLD only n=44 counseling n=18 number of Tx over 1yr varied	Bilateral limb TCvol, CM wrist to axilla at 4-cm intervals *Measurements*: pre & post Tx	Improvement CDT: 55.7% * MLD: 41.2% * Counseling: 24%*
Maher et al. (2012)	n=30	MLD n=15 MLD + CS n=15 One session	Perometry, BIS interlimb ratio- pre, post:30min, 60min, 120min	No significant change for either
McNeely et al. (2004)	n=50 RCT	2 groups CB n=25 MLD/CB n=25 4 week treatment	Bilateral limb water displacement volumetry & TCvol, CM from the wrist to axilla at 4-cm intervals *Measurements*: pre, 1, 2, 3, & 4 weeks post tx	Both treatments arm vol ↓ significantly* Patients with mild LE had significantly* larger vol reduction with MLD/CB

Reul-Hirche et al. (2011)	n=160 Breast cancer survivors RCT	MLD & education n=79 Only education n=81 MLD: 40 sessions over 20 wks	Water displacement, HRQoL Baseline & 1,6,9 &12mos	No difference in incidence of LE at 3,6,& 12 months
Sitzia et al. (2002)	n=28 RCT	MLD n=15 SLD n=13 2 week treatment	Bilateral limb, TCvol, CM at 2cm intervals Measurements: pre & post Tx	% change in excess limb vol. MLD 33.8% SLD 22%
Vignes et al. (2006)	n=356 Prospective cohort	All had CDT Maintenance at 12 months MLD n=246 CB n=263 CS n=322	TCvol,CM from the wrist to axilla at 5-cm intervals, Measurements: pre & post CDT and 6 &12mo post CDT	CDT: vol ↓ significantly* 12mo Noncompliance to CB & CS risk factors for arm vol ↑ significantly* 12mo Noncompliance to MLD not a risk factor
Vignes et al. (2011)	n=682 Prospective cohort	MLD CS MLD +CB MLD +CS	Bilateral limb TCvol CM wrist to axilla at 5-cm intervals Hazard ratio: calculation of arm edema vol ↑ ≥50% Measured at 1,2 ,4 yrs	After 12mos Hazard ratio MLD: 1.91* CS: 0.65 MLD + CS: 1.09 CB + CS: 0.53* MLD + CS+CB 0.73 ∴ Better without MLD
Wilburn et al. (2006)	n=10 prospective randomized crossover study	Flexitouch™ 14day Tx SLD 14day Tx 7 day washout w/ garment	Bilateral limb TCvol CM wrist to axilla at 4-cm intervals Measurements: pre & post Tx	Flexitouch™**:** significant* volume reduction SLD**:** no significant volume reduction
Williams (2002)	n=29 Randomized crossover study	SLD 21day Tx MLD 21day Tx 42day washout w/ garment	Measurements: pre & post each Tx Bilateral limb TCvol, CM at 4-cm intervals Modified skinfold calipers Ultrasound, QLQ C30	MLD: vol ↓ significantly* SLD**:** volume ↓ not significant Excess vol difference between MLD vs SLD not significant Trunk edema & QoL no significant difference MLD: significant*↓ discomfort, pain, heaviness, fullness

ALT: Aqua Lymphatic Therapy **CB**: multi-layered compression bandaging; **CDT**: combination of MLD, compressive garment, exercise instruction and skin care **CM**: circumferential measures; **CS:** Compressive sleeve; **CSER:** Casley-Smith exercise routine; **Hazard ratio (HR)**: LE edema volume increase of ≥ 50%; **LE:** lymphedema; **MLD**: Manual Lymphatic Drainage; **SLD**: simple lymphatic drainage (self massage); **SM**: self management; **SP (**standard physiotherapy): bandage, elevation, head–neck, shoulder exercises and skin care; **TCvol**: Truncated cone volume calculation

These findings show MLD has demonstrated effectiveness for reducing limb volume in the short term; however, MLD alone is not a replacement for standard care (e.g., compressive bandaging, garments and exercise), and it would appear from the studies in this review of literature, that standard care appears to be an effective modality without MLD. In conclusion, the efficacy of these three treatment interventions is not well established in the literature and there is a pressing need for more quality studies to establish a well founded base of knowledge for clinicians to draw from when creating treatment plans for women with BCRL. Although the studies mentioned gave evidence of immediate results, there has not been a comprehensive study that has recorded the 24-hour residual effect of all of these three treatment interventions in the same cohort of women with Stage II BCRL

CHAPTER 2: RATIONALE AND OBJECTIVES OF THE STUDY

In Canada, 23,000 women are diagnosed with breast cancer each year (Statistics, 2012). Approximately 30% of those who survivor the cancer will develop BCRL as a sequelae to the cancer treatment. In Quebec 5,500 women are diagnosed with breast cancer each year (Statistics, 2012). For these women, most lymphedema treatments are not covered by the healthcare system and the high cost of "usual care" (e.g., MLD or compressive bandaging and/or compression garments) can make the treatment prohibitive for many of these patients.

In spite of the education and advice concerning the maintenance phase of patients diagnosed with BCRL, some patients have had difficulty adhering to a maintenance program resulting in a return to the limb volume of their lymphademetous arm before the Phase I treatment (Johnstone, 2006). Another complication patients face in adhering to their maintenance program is the fact that some suffer adverse effects from wearing compressive garments such as hand swelling as well as skin irritation and pain from continuous rubbing and friction from the garment seams (Vignes S., 2009). These factors highlight the challenges and importance of determining the most effective means for maintaining or reducing limb volume reduction for women with BCRL.

The three interventions have shown immediate and positive improvements for BCRL over the short term (Bracha, 2010), (Tidhar D., 2010), (McNeely, 2004). Whether the impact of ALT, MLD, and CSER bestow immediate effects that last over a 24hrs period remained to be seen. If our investigation revealed that any one of the 3 interventions provided a daily positive influence on maintaining or reducing limb volume, this might indicate a decreased need for the use of the compressive garments. This would lead to fewer physical, psychological and social side effects from reduced garment use and would provide a substantial savings in the purchase of these

garments for the lifelong maintenance of BCRL. Secondly, if the results of either ALT or CSER could provide evidence that either intervention is as effective as MLD, then patients will be able to make an informed choice before spending $80-$100 for a treatment for their lymphedematous arm. Most importantly, there exists the possibility that a specialized daily exercise routine or aqua-therapy could give a woman with BCRL added symptom relief and improved quality of life for very little cost if any.

Clearly, further research is needed to present evidence in defining the most effective treatment available for limb volume reduction and maintenance for this lifelong condition.

Research questions, hypotheses and study objectives

Research Questions**:** The following questions have been proposed: 1) Of the three treatment interventions being studied which one is the most effective in promoting limb volume maintenance or reduction? 2) Will there be any new or changes in physical symptoms of the affected extremity following anyone of the treatment interventions? 3) Which one of the three interventions do the women prefer?

Study Objectives**:** 1) To assess the immediate and short-term effects on ECF volume following a single bout of activity from three different interventions for BCRL (e.g., MLD, ALT, CSER), 2) To determine which of the three interventions is most effective in maintaining or reducing limb volume over a 24 hour period, 3) To record any changes in physical symptoms after each treatment and, 4) To ascertain which of the three modalities the women prefer.

Hypotheses

Based on the theory of the mechanisms at work with CSER and ALT and evidence that indicates muscle movement encourages lymph flow, we hypothesize that CSER and ALT will

have some efficacy in reducing the arm volume but, to what extent and duration is yet to be seen.

The conflicting evidence on the efficacy of MLD leads us to hypothesize that MLD will have some immediate effectiveness in volume reduction but will not demonstrate any lasting effects at 24 hrs post-intervention (Velanovich, 1999).

If there are any changes in symptoms such as decrease in heaviness or aching they will correlate positively with volume reduction. Therefore, we hypothesize that there will be immediate positive changes in symptoms for all three interventions that may last 24 hrs for the ALT and CSER but, these positive changes will not last with the MLD intervention.

CHAPTER 3: METHODS AND PROCEDURES

Experimental Design

This project was a repeated-measures crossover design that incorporated both multiple measures over time and 3 different levels of the independent variable. The advantages of this design include the need for fewer participants to increase statistical power as the participants acted as their own control. This condition also decreased the potential effects of natural variation. Specific weaknesses of this type of experimental design includes the possibility of the natural progression of lymphedema over the 5 weeks could be a potential threat to the internal validity of the design. Another disadvantage to this design could relate to the feasibility of participant recruitment and adherence due to the extended time commitment. For this repeated measures design all participants completed one 45min session for each of the three interventions over a five week period with a two week “washout” period in between interventions. An important consideration listed in the inclusion criteria for recruitment was that all the women recruited have Stage II lymphedema. Recruiting only participants with Stage II was crucial to limit the between participant variability.

Approval for the study was obtained from the McGill University Health Center’s Research Ethics Board. This research was carried out in accordance with the Canadian government’s Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans and the Helsinki Declaration of 1975, as revised in 2008.

Participant recruitment: All eligible patients were invited to participate and 19 of the 35 women who were found to be eligible for the study were recruited consecutively at the McGill University Health Center (MUHC) Lymphedema Clinic. All women who were recruited read and signed an informed consent document approved by the MUHC. The recruitment was difficult as is evident

in the 54% recruitment rate of the 35 potential patients. The adherence rate was 84%, three participants dropped out after receiving one intervention each. All questionnaires and informed consent documents were duly translated into French.

Inclusion Exclusion Criteria

Inclusion Criteria:

- Women age 35-75
- BMI ≤ 45 kg/m²
- 6 months or more post-breast cancer treatment
- Breast cancer stages I-III in remission
- Stage II unilateral BCRL (≥10% limb volume difference)
- Having completed the intensive phase of CDT
- Willing to refrain from wearing a compression garment for 24 hours prior to and following each intervention (for a total of 48 consecutive hours) on three occasions over a five week period
- Not averse to entering a swimming pool

Exclusion Criteria

- Active cancer or significant chronic illness
- Pre-existing heart condition (e.g. pacemaker, congestive heart failure, hypertension, peripheral artery disease)

- Pregnancy (>24weeks)
- Treatment for lymphedema in the last two weeks (e.g. MLD and/or compressive bandaging)

Patient demographics: During the initial visit a brief questionnaire (Appendix A) was administered to ascertain dates and types of treatment for breast cancer, date of BCRL onset and stage, which arm was affected and which was the dominant upper extremity. Any missing data was retrieved from the patients' medical files of the MUHC Lymphedema Clinic with permission granted by the participant. For details of participant characteristics, see Table 6

Interventions

The following is a description of the three treatment interventions including a brief discussion of the postulated mechanisms of action.

Aqua lymphatic therapy (ALT): is a gentle form of aqua therapy performed by the patient as part of a class or individually in a warm shallow swimming pool with a depth of 4 to 4.5 feet (1.2 to 1.4 m) and at a water temperature of 88 to 91.5°F (31-33° C). Women are not permitted in the pool if they have an active skin infection. Standing in the pool with the water at neck level the ALT session begins with chest breathing exercises attempting to clear the thoracic duct. The exercises are performed slowly, from proximal to distal, with the arm position taking advantage of the natural hydrostatic pressure gradient of the pool. Thus, the exercise period begins with the arms floating (15-20 minutes) and finishes with the arm vertically submerged in the water (15-20 minutes). The exercise sequence involves the shoulder girdle, abdomen, head and neck, and then the distal arm. Movements are used to clear the axillary lymph nodes, followed by side bending with hand pointing down to activate the latissimus dorsi and quadrates lumborum

muscles for proximal activation of lymph flow. The exercises end with dolphin type diving, arms extending in front for distal to proximal water massage. The limb position and direction of movement during the exercise encourages lymph flow away from the affected arm and causes a massage effect of the limb. Finally, deep breathing and self-massage or massage by a fellow participant are incorporated several times to augment the effects of the exercises at specific times during the sequence.

The suspected mechanisms behind this treatment are threefold: incorporating water's hydrostatic property to create a pressure gradient enhancing directional lymphatic flow; the viscosity of water providing resistance with movement for a gentle massage or; more vigorous movements in specific directions for strengthening and encouraging the extrinsic pumping of the lymphatic vessels by adjacent muscle movement. The sequence of movements is designed to initially encourage proximal clearing of lymph followed by distal to proximal movement of lymph. Theoretically, this combined movement is expected to lead to the reduction or maintenance of limb volume (Tidhar D., 2010). The authors also highlight the benefits of the group activity format and its social and self-efficacy benefits. For this study this intervention was lead by a lymphedema specialist with over 7 years of experience leading ALT classes.

Manual lymphatic drainage (MLD): a method of massage for lymphatic drainage was developed and first presented at a conference in 1936 by Emil Vodder (Williams A. , 2010). This method of massage stretches the skin and incorporates gentle rhythmic circular movements in specific directions, interspersed with rest periods, using an approximate pressure of 30mmHg which varies according to the edema (e.g., increased pressure for breakdown of fibrotic tissue). The suspected mechanism to encourage lymph flow with this technique is threefold: 1) moving the lymph with slowly increasing pressure; 2) an attempt to create pumping by the rhythmic

pressure, rest (no pressure) movements; and 3) an attempt to stimulate lymphatic vessel contraction with the stretching of the skin. The stroking technique used in MLD is proposed to act as an analgesic. This is based on the neurophysiology theory that the stimulation of the unmylinated tactile afferents, by stroking the skin, has been shown to inhibit nociceptive signaling (Olausson H., 2010). The massage treatment begins proximally at the root of the limb and then moves in a distal to proximal direction. The massage finishes by moving from the axilla progressively distal. Often the thorax and the opposing limb are treated as well. A single treatment lasts approximately 45 minutes (Kasseroller, 1998). The MLD therapist that treated all the participants for this research had over 4 years of experience and had been recently recertified by the Dr. Vodder School.

Casley-Smith exercise routine (CSER): is a gentle (low intensity) 45 minute exercise routine that incorporates breathing, slow rhythmic mild isotonic muscle contractions of the affected arm muscle interspersed with self-massage to promote regional lymphatic fluid movement and venous outflow (Casley-Smith J.R, 1998). The routine begins with deep breathing to facilitate clearing of the thoracic duct and proximal muscle group exercises; followed by distal muscle group and concluding with proximal muscle group exercises. The session ends with a period of relaxation. The proposed mechanism for this method involves the systematic application of varying pressures through movements to specific areas with the intent to create a pressure gradient to encourage the directional flow through the lymphatic pathways. The muscle contractions are believed to facilitate extrinsic pumping of the lymph through the vessels. For example, clearing of the thoracic duct is thought to be accomplished using deep breathing with synchronized chin curls and flexion/extension of the upper spine. Thus, applying varying pressures to the thoracic area, utilizing extrinsic pumping actions of the lymphatic vessels encourages the clearing of lymph from the thoracic duct into the great veins. Beginning the

routine proximally (with lymphatic trunks and nodes) is performed with the intent to overcome the hydraulic resistance in the nodes that is thought to slow post-nodal lymph flow (Havas E P. T., 1997). The exercise trainer leading this intervention during this study had been trained by Judith Casley-Smith the developer of this exercise routine.

To minimize a possible sequencing effect participants were assigned to receive the interventions (A=ALT, B=MLD, C=CSER) in one of the following five different sequences:

A in period 1, B in period 2, C in period 3 (one participant),

A in period 1, C in period 2, B in period 3 (three participants),

B in period 1, A in period 2, C in period 3 (six participants),

C in period 1, A in period 2, B in period 3 (two participants),

C in period 1, B in period 2, A in period 3 (four participants),

There was a “washout” period or delay between each intervention of a minimum of 10 days.

Outcome Measures

The pre-intervention symptom questionnaire was administered and bilateral limb volume measurements were taken immediately prior to receiving each intervention. The post-intervention assessments were done at 10min, 60min and 20-24 hrs. Questionnaires: The Lymphedema Breast Cancer Questionnaire (Appendix B) was given before the first treatment intervention and an abridged version for self-reporting of symptoms (e.g., heaviness, tightness, numbness, and aching) was given immediately prior to the other two interventions. After the final intervention a structured three-question questionnaire (Appendix C) on intervention preference was administered.

Anthropometry: Body weight was measured to the nearest 0.1 kg using a digital scale (Amcells TBS Series, USA). Height was measured without shoes to the nearest 0.5cm using a mechanical stadiometer (Seca). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared (kg/m²).

Bilateral changes in limb volume were measured using two methods:

1) Bioelectrical impedance spectroscopy (BIS). Participants were in a supine position on a non-conductive table with their arms extend slightly abducted, palms down, and with legs slightly apart. The skin was wiped with an alcohol swab and allowed to dry. Four electrodes were applied to the skin in the following locations: a current source electrode on the dorsal surface of the third metacarpal of the measured arm, and current sink on the third metatarsal of the foot of the measured side. Voltage sensing electrodes were placed on the dorsum of both wrists. With four leads attached to the electrodes, a small constant AC current (200μA) was passed from hand to foot and the drop in voltage was measured. The measures of impedance (opposition to the flow) of the electric current through the body fluids are used to calculate the volume of extracellular and intracellular fluid (Ward, Czerniec, & Kilbreath, 2009). BIS has accurately detected early stage lymphedema and has been used to monitor treatment efficacy (Cornish, et al., 2001). Most recently, BIS device used in this study has been shown to be a highly precise instrument and sensitive enough to detect as low as a 44ml change in ECF within the lymphedematous arm (Newman A, 2013). The BIS system used in this protocol was the ImpediMed SFB7. For the analysis, the system's software (Bioimp version 2.25, ImpediMed Ltd., Brisbane, Australia) was used with the parameters set as follows: a data rejection level of "none" (default setting) and an operator-chosen frequency range of 3 kHz-500 kHz. These have been determined to be the most appropriate settings for this population (Newman A, 2013).

2) Circumferential arm measures (CM). Using a standard tape measure, circumferential measures from the wrist to axilla at 10-cm intervals were obtained. Arm volumes were calculated determined by summing segment volumes derived from the truncated cone formula. CM is a commonly used clinical method for monitoring limb volume in the treatment of lymphedema and has been found to be highly reliable and correlates well with water displacement which is the currently accepted "gold standard" for limb volume measurement (Gjorup C, 2010), (Czerniec S. A, 2010), (Karges, 2003). The unaffected limb served as an internal control. Bilateral measurements were taken before each intervention and at 10 min, 60min and 20-24 hours post-intervention by the author who was blinded to previous measurements by using a new data collection sheet for each set of measures. The author administered all questionnaires. The author had had three years of experience as a research assistant performing assessments with the aforementioned measurement tools.

Statistical Analysis

The statistical package used was NCSS Statistical Software (version 7.1.17; Kaysville, Utah). The analysis done was a 3 (interventions) x 4 (time periods) repeated measures analysis to estimate the variance among the volume measurements for each of the three treatment interventions. A $p \leq 0.05$ was used to determine if the variance estimate was considered to be statistically significant. When a p-value of ≤ 0.05 was obtained, the Tukey-Kramer Multiple-Comparison post-hoc test was run to determine significant differences between individual means. The quantitative analysis of the LBCQ questionnaires was performed using a χ^2 (Chi squared) test. For qualitative analysis we intend to perform a structured questionnaire content analysis with answers grouped by themes.

CHAPTER 4: RESULTS AND DISCUSSION

Results

Within group differences: There were no significant changes from baseline ECF at any of the measured time points (10 min, 60min, 24 hrs) following MLD or CSER. Of the three interventions, only ALT had a statistically significant ($p \leq 0.05$) change from baseline in ECF volume (26.9mls) at the 24-hour post-intervention measurement period (Table 7).

Between group differences: In comparing the ALT vs MLD interventions from baseline to 20-24hrs, ALT has a 3.3% greater ($p=0.038$) mean percentage ECF volume change than MLD at 20-24 hours (Table 8). No other ECF differences exist between ALT vs CSER or MLD vs CSER. With respect to circumferential arm measurements, there was a significant difference ($p=0.021$) in the percentage change between ALT and MLD at 10 min post intervention. No other circumferential arm measurement differences exist between ALT vs CSER or MLD vs CSER.

There was a significant ($p \leq 0.05$) main effect between ALT and MLD. Post-hoc analyses revealed differences in ECF with significantly greater volumes for ALT than MLD at baseline ($p=0.016$), 10 minutes ($p=0.022$) and 60 minutes ($p=0.009$) post-intervention (Table 8). Mean ECF volume for CSER was significantly less than ALT at 10minutes ($p=0.004$) and significantly lower than MLD at 60 minutes ($p=0.004$).

LBCQ and subjective preferences

The results of the abridged LBCQ questionnaire of symptoms showed that for symptoms experienced during assessments, there were no significant differences between groups. The two most common symptoms reported in all three intervention sessions were swelling and heaviness. There was no clear consensus on the most preferred choice of intervention;

however, the majority (13/16) of participants preferred ALT (7/16) and MLD (6/16). The treatment that participants thought to be the most effective subjectively was ALT (10/16).

Discussion

There were two main objectives to this study. First, we wanted to determine if there would be a detrimental effect on limb volume (e.g. significant increase in ECF measured by BIS and limb volume by CM) following a single bout of each intervention treatment in women who had their compression garments removed for 48 hrs. Secondly, we wanted to determine which of the three interventions if any, is most effective in reducing limb volume in the immediate (10-60 min) and short term (24-hour) post-intervention time period. The results of this study suggest would tend to suggest that the ALT intervention was the most effective of the three interventions in terms of significantly reducing ECF volume not only over time but also in comparison to the MLD intervention. In comparison, an earlier study involving ALT, Tidhar et al. (2010) had immediate mean volume reduction of 53.5ml immediately following the first ALT session, measuring with water displacement. With respect to MLD, post-intervention limb volume did not change. Although it is tempting to state that MLD maintained limb volume constant over time; however, without the comparison of a control group, we cannot equivocally state that MLD had any effect whatsoever. In agreement with our immediate findings for MLD, a study by Maher et al. (2012) where they combined MLD & compressive sleeve and compared it with MLD in a one session design measuring with BIS and perometry at 30min intervals for 2hours found there was no change in volume for either intervention. Similarly, CSER did not produce any ECF volume change at any of the post-intervention time points. Despite these findings with CSER, it is encouraging from a clinical perspective to observe that ECF did not increase in the short- term post intervention period. Similarly, a study by Moseley et al. (2007), where the intervention was a 10 minute gentle arm exercise with deep breathing, their findings indicated an immediate

median reduction of arm volume of 52mls (p=0.004) that was not sustained at the 60min mark where the median volume returned to baseline.

Whether or not ALT or CSER will have a greater impact over the longer term (e.g. 6-8wk "training effect) remains to be seen. With respect to lymphatic system adaptations two studies investigating lymph clearance rates (CR) utilizing lymphoscintigraphy to measure lymphatic function at rest and during exercise found similar results. In the first study, Lane et al. (2007) divided the participants in to three groups 1) breast cancer survivors with BCRL (n=10), 2) breast cancer survivors with no LE (n=10) and 3) healthy controls (n=10), and implemented moderate intensity upper body exercise as an intervention and found similar increases in lymph clearance rates (CR) in the upper limbs for all three groups. Havas et al. (1997) demonstrated that along with increased CR of interstitial fluid during exercise, endurance trained healthy subjects had higher resting interstitial fluid CR than that found in the untrained healthy subjects. Consequently, the authors suggest that exercise may produce adaptations of the lymphatic system. Although there appears to be a training induced increase in the resting CR of the interstitial fluid, it is still unclear if theses changes are due directly to morphological changes in the lymphatic system.

Limitations:

As mentioned previously, there was no control group included in the design of this pilot study. Since this was designed to be a pilot study, we knew that the sample size used would underpower the study and prevent us from making any significant generalizations about our findings. In fact, one of the secondary hypothesis to this pilot study was to determine what sample size would be needed in order to adequately power the follow up study. By calculating standardized difference = target difference / standard deviation and referring to a nomogram

produced by Altman (Altman, 1991), we are able to determine that it would require 50 participants to reach a suitable level of power (0.8).

Normally, we would not have expected the baseline ECF volume measurements to be any different from one another prior to each intervention. However, this was not the case for the baseline ECF measurement prior to ALT which was statistically higher that the baseline MLD value. In a previous study involving ALT Tidhar et al. (2010) found that there was no correlation between initial arm edema and the reduction in the volume after the ALT intervention (linear correlation of 0.3, $p>0.2$). Perhaps the difference in baseline volume was related to the women refraining from wearing their compression garment or weather conditions it is difficult to know. Since the baseline measurements for ALT were done in a public pool setting, perhaps the hot humid environment of the public pool had a vasodilatory impact on the lymphedematous arm thereby increasing the volume of the ECF compartment. In a comparison of the two interventions (ALT & MLD) for the baseline ECF measures of the non-affected (NA) arms we found the mean ECF volume for ALT was 19ml greater than for the MLD mean baseline measure. Although a smaller magnitude of difference it does suggest that the ECF volume of the NA arm may have also been affected by the pool environment.

It is important to note that none of the volume changes measured throughout this study exceeded the least significant change of 43.7ml for BIS measures of LE arm. This value of 43.7ml was derived as a result of our precision calculations in the included manuscript (Determining the precision of dual energy x-ray absorptiometry and bioelectric impedance spectroscopy in the assessment of breast cancer-related lymphedema). The least significant change (LSC) is the smallest change between 2 measurements taken over time that must be

exceeded for the change to be considered true with 95% confidence. Therefore we cannot state with confidence that the changes measured in this study exceeded measurement error.

Another factor that may have influenced the effects of all of these interventions is the long standing lymphedema condition in many of these women, as indicated by the average time since lymphedema onset of 6.5 years. This evidence of chronicity along with only six of the participants reporting pitting suggests that the composition of many of the women's LE limbs may have developed fibrotic tissue and excessive adipose deposits impacting the reducibility of the arm volume with these interventions.

The time commitment for participants in this study was approximately 12 hours over a minimum of 5 weeks. Several potential participants declined to participate indicating they found the time commitment too onerous. This factor along with the physical activity requirement to fulfill the protocol may have deterred less motivated and/or less physically active LE patients from participating and therefore an overall recruitment bias may exist. Another limitation of this study is the lack of controls or accounting for participants' daily activities during the 24 hour period immediately following the intervention before the final assessment. The activities of the 16 women who participated in this study, during the 24hr period between assessments varied from staying at home resting, to assisting family in the hospital, to a day of physical work.

Recommendations for future research:

Although the results of this pilot study may lead to more questions than answers and do not allow any clear conclusions to be drawn, this study has set the stage for future studies on the topic. Studies to, investigate these same research questions with a greater sample size and more control over variables appear warranted. Another important study that is needed would be tracking ECF volume changes over longer periods for ALT and CSER with and without

compressive garments. A study design to build upon would be the research of Vignes et al. (2010) replacing MLD with ALT and or CSER; in their prospective study measured 4 groups over a four year period. The groups were 1) MLD, 2) compressive sleeve (CS), 3) MLD & CS and 4) MLD & compressive bandages (CB). As well more investigations into the physiological effects of exercise on the lymphatic system are required.

CHAPTER 5: MANUSCRIPTS

The first of the two manuscripts contained in this chapter of the thesis (Determining the precision of dual energy x-ray absorptiometry and bioelectric impedance spectroscopy in the assessment of breast cancer-related lymphedema) is based on a research project in which this author was involved in recruiting, organizing logistics, performing all measurements and data collection. This manuscript is a copy of an article published in Lymphatic Research and Biology

The research questions for this study are: 1) Are DXA and BIS technologies feasible assessment tools to measure tissue composition of lymphedematous upper limbs as diagnosed in the BCRL patient population? 2) Is excess fat mass present and detectable by DXA in affected upper extremities of patients with Stage I and/or Stage II lymphedema? 3) What is the precision of DXA in measuring fat mass in the upper extremities in this population? 4) What is the precision of BIS in detecting extracellular fluid in stage 1 and/or stage 2 lymphedema? In the reporting of the results from this research this manuscript goes into detail about the measurement protocols, the calculations involved in the derivation of arm and ECF volume as well as the precision of both BIS and CM.

Being involved in this first research study allowed the author to gain familiarity with this specific patient population of women with BCRL and furthered the author's experience with the required measurement tools (e.g. BIS and CM).

Manuscript 1

Determining the precision of dual energy x-ray absorptiometry and bioelectric impedance spectroscopy in the assessment of breast cancer-related lymphedema

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Abstract

Background: The composition of breast cancer-related lymphedema (BCRL) has been shown to evolve from the initial accumulation of fluid to the development of fibrotic lesions and abnormal fat deposition. Therefore precise and reliable assessments of BCRL are required to develop accurate staging and management. Although dual energy x-ray absorptiometry (DXA) and bioelectric impedance spectroscopy (BIS) have been used to assess BCRL no study has evaluated the precision of these two modalities in the same cohort. *Methods and Results*: We determined the precision of DXA and BIS in lymphedematous (LE) and non-affected (NA) arms of 24 women with Stage II unilateral BCRL. Precision was calculated from the results of paired bilateral arm measurements obtained from DXA scans measuring fat, lean and bone mineral masses, BIS measuring extracellular fluid (ECF) and total fluid volume and circumferential tape measurements (CM) of the arms to calculate the anatomic volume. Precision error was expressed as the root mean square (RMS) of the coefficients of variation (%CV) and standard deviations (SD). *Results***:** The precisions of DXA and BIS varied from 1.16% (DXA measurements of LE arm total volume) to 1.86% (BIS LE arm total fluid volume) and from 0.95% (DXA lean mass of NA arm) to 1.72% (DXA BMC of NA arm). Precision of CM measures of arm volume were 1.71% CV for LE arm and 2.51% CV for NA arm. The fat and lean masses of the LE arm exceeded the NA arm by about 15% ($p < 0.0001$). ECF and total fluid volume of LE arm was 22.6% and 19% greater than the NA arm ($p < 0.0001$), respectively. *Conclusion*: For BCRL, these findings suggest that DXA and BIS are two measurement instruments that provide acceptable levels of precision for the measurement of arm lean mass, fat mass and ECF volume, respectively.

Key words: breast cancer, lymphedema, DXA, BIS, precision.

Abbreviations:

BCRL	breast cancer related lymphedema	ECF	extracellular fluid
BIS	bioelectric impedance spectroscopy	LE	lymphedema
BMC	bone mineral content	LSC	least significant change
CM	circumferential measurements with tape measure	NA	non-affected
DXA	dual energy x-ray absorptiometry		

Introduction

Breast cancer-related lymphedema (BCRL) can be mild to severe but often presents as a chronic, progressive multi-stage condition that is initially diagnosed by swelling of the arm(s) and/or the hand(s). The swelling is typically caused by an accumulation of protein-rich interstitial fluid that has been shown to occur in approximately 25% of women following surgical removal and/or radiation of the axillary lymph vessels and nodes (Petrek, Senie, Peters, & Rosen, 2001). BCRL can cause physical and psychological distress and negatively affects work and social functioning (Thomas-MacLean R, 2008), other clinical symptoms include pain and shoulder range of motion restrictions which have been shown to affect over 50% of breast cancer survivors (Lawenda, Mondry, & Johnstone, 2009). It is believed that the treatment-induced damage to the axillary region inhibits the clearing of intercellular fluid by the lymphatic system to the venous system thus exacerbating the lymphedematous condition (Devoogdt, Van Kampen, Gearts, Coremans, & Christiaens, 2010). Over time, the accumulation of lymph and the subsequent swelling may compromise normal tissue oxygenation as well as muscle and immune system response; predisposing the area to recurrent infections. Also associated with

chronic inflammation is an excess of fibroblasts and collagen deposition which lead to fibrotic lesions that may be found within the edematous tissues (Tassenoy A, 2009). Although not yet completely understood impairments to the lymphatic vessels coupled with chronic inflammation appear to alter the lymphatic system's role in fat metabolism, which may result in the deposition of abnormal amounts of subcutaneous fat in lymphedematous arms (Alitalo, 2011). This progressive development may in fact compromise and challenge the current paradigms for assessment and treatment of BCRL.

Current decongestive therapies (e.g. compressive bandaging, manual lymphatic drainage and remedial exercises) aid in the elimination of excess fluid in the extracellular fluid (ECF) space however, with the more advanced stages of BCRL, the standard decongestive therapies may prove to be ineffective in reducing the cumulative fat and fibrosis (Vignes S, 2006), (Armer & B.R., 2005). Unless other innovative forms of therapy are introduced, the advanced stages of BCRL will remain a chronic incurable condition (Cornish, et al., 2001), (Bar Ad, et al., 2010), (Piller & Carati, 2009) (Lymphology, 2009). Before other forms of therapy and treatment are even considered, clinicians and health care providers must be capable of discriminating and assessing the difference between the fluid and non-fluid (e.g. total arm mass including bone) compartments. Some of the most frequently used clinical methods (e.g. circumferential measurements and volume displacement of the arm) of assessment of BCRL fail to provide the sensitivity or are unable to differentiate between tissue types as well as fluid and non-fluid compartments. Having access to this information would assist in directing treatment plans. Techniques such as dual-energy x-ray absorptiometry (DXA) and bioelectric impedance spectroscopy (BIS) are two measurement devices that are gaining prominence and acceptance in the lymphedema research community with regards to their safety, ease of measurement and ability to estimate soft tissue and fluid components, respectively (Gjorup C, 2010), (Czerniec S.

A, 2010), (Paskett E.D, 2012), (Armer & B.R., 2005).There is little published information on the precision of these two instruments for edematous upper extremities, particularly when they are both derived from one cohort of women on the same day. The precision is important in that it defines the least significant change (LSC) in tissue composition and fluid levels in the affected arms of women as their lymphedema condition changes with treatment and over time. Furthermore, different forms of treatment therapies and interventions throughout the progressive stages of BCRL would necessitate the repeated use of DXA and BIS in the same patient cohort. Thus, the precision of DXA and BIS is absolutely essential in order to determine the true effectiveness of any treatment or intervention. Therefore, the aim of this observational pilot study was to determine the precision of the DXA and BIS devices and their clinical feasibility in estimating fat and lean masses and fluid volumes in lymphedematous (LE) and non-affected (NA) arms.

Materials and Methods

Between June 2010 and November 2011, 40 eligible patients visiting the Montreal General Lymphedema Clinic were invited to participate in this study. Twenty-four women with a clinical diagnosis of unilateral Stage II lymphedema were recruited from the clinic and written informed consent was obtained. Eligibility criteria for the study included: 1) females between 40-70 years of age, 2) BMI ≤ 30, 3) six months or more post-breast cancer treatment, 4) breast cancer Stages I-III in remission, 5) no significant chronic illness (e.g., congestive heart failure), 6) with unilateral Stage II lymphedema, 7) not pregnant, and 8) no history of involvement in sport involving unilateral upper extremity (e.g. tennis). Staging was determined according to the International Society of Lymphology criteria (Lymphology, 2009). The staging assessments

were performed by the clinic's physician and physiotherapists who are all lymphedema specialists.

All assessments took place at the McGill Nutrition and Performance Laboratory in Montreal. Participants were 57.2±7.8 years of age, 70.1±6.3kg in weight, and 162.1±6.7 cm in height and had a BMI of 26.7±2.6 kg/m². All values are expressed as mean ±SD. Body weight was measured to the nearest 0.1kg using a digital scale (Amcells TBS Series, USA) and height was determined without shoes to the nearest 0.5cm using a wall mounted stadiometer.

Total body DXA scans were performed with a Lunar Prodigy Advanced scanner (GE Healthcare, Madison, WI, software version 2006). Prior to each assessment session the measurement stability of the scanner was documented using the manufacturer supplied aluminum spine phantom. From the total body scans appendicular fat, lean and bone mineral content of both arm segments were identified and calculated with the region of interest extending from the gleno-humeral joint to the finger tips. Each measurement session began with two consecutive total body DXA scans with the patient supine on the DXA table. After the first scan the patient stood up and then was repositioned on the table for the second scan. Each scan took approximately 8-10 minutes. Using previously derived densities for: fat, (0.9gm/ml); lean mass, (1.1gm/ml); bone mineral, (1.85 gm/ml) the measured DXA tissue weights were transformed into estimated volumes using the following equation: v= m/d where v = volume, m = mass and d = density (Gjorup C, 2010), (Brorson H O. K., 2009).

For the BIS (Model SFB7, ImpediMed Ltd., Brisbane, Australia) measurements, each participant laid supine on a non-conductive surface, with all limbs slightly abducted. The skin surface at the sites of electrode placement was thoroughly cleaned with alcohol swabs and 4 disposable dual tab electrodes were accurately placed with reference to anatomical markers. One dual tab

electrode was placed at each wrist next to the ulnar head extending to the dorsal surface of the hand, 1-2cm proximal to the metacarpo-phalangeal joint of the middle finger. The remaining two dual tab electrodes were placed at the ankle joint of each leg between the medial and lateral malleoli to the dorsal surface of the foot, 1-3cm proximal to the metatarso-phalangeal joint of the second toe. These locations were selected according to the manufacturer's recommendations. A small constant current (200μA) was generated and passed between the electrodes spanning the body and the voltage drop between the electrodes provided a measure of impedance. The BIS measurements of both arms and total body took approximately 8-10 minutes. Ten minutes later after standing and removal of the electrodes, the patient was repositioned on the non-conductive surface and a new set of electrodes were placed in the identical location as before. The BIS procedure was then repeated. Before each session a system test was run as per manufacturer's recommendation. Participants were asked to fast and refrain from consuming any fluids for 4 hours prior to their appointments.

The BIS system's analysis software (Bioimp version 2.25, ImpediMed Ltd., Brisbane, Australia) fits a semicircular locus to the reactance vs resistance data at each frequency to give an estimate of resistance at a frequency of zero and infinite. For this analysis, the parameters were set as follows: a data rejection level of "none" (default setting) and an operator-chosen frequency range of 3 KHz-500 KHz was selected. This frequency range was chosen, after visual inspection of the first eight data sets and the frequency range adjustments revealed that 3 KHz-500 KHz produced better curve fits of the data to the theoretical semicircular locus in all cases.

The utility of the resistance values measured by BIS lies in the theory that at low frequencies the cell membrane is highly resistant thus; the electric current travels in the extracellular fluid surrounding the cells only whereas, at high frequencies the cell membrane is less resistant, and

the electric current travels in the intra and extracellular fluid and thus resistance estimated at a frequency of zero (R_o) represents ECF and resistance estimated at a frequency of infinity (R_∞) represents total fluid (Cornish, et al., 2001). Using the method published by Ward, Czerniec, & Kilbreath (Ward, Czerniec, & Kilbreath, 2009), the fluid volumes for arm ECF and arm total fluid were derived from the equation: V(volume) = p (arm L^2/R), where p= coefficient of resistivity, R = resistance (R_o or R_∞) and L = length of arm, (where arm length was calculated as a proportion of height).

Paired bilateral arm volume measurements were obtained by serial circumferential measurements (CM) to the nearest millimeter with a retractable no stretch soft tape measure (Juzo, Cuyahoga Falls, OH). The CMs were taken at the following five points on the arm: 1) the mid-point of the ulna styloid, 2)10cm proximal to the ulna styloid, 3) antecubital fossa, 4)10cm proximal to the antecubital fossa and 5) level with the axilla. The volume of each segment or truncated cone was derived from the following expression: $V = l (C_1^2 + C_1 \cdot C_2 + C_2^2)/37.7$, where l is the length of the segment, C_1 and C_2 are the circumferences of each end of the segment. The total volume of the arm was the summation of these segments (Taylor R, 2006).

Each measurement session consisting of paired measures of all three measurement techniques and lasted approximately 60 minutes. All measurements with the DXA and BIS devices and the tape measure arm circumferential volume determinations were performed by one of the authors (AN) who had had two years of experience in the use of all three measurement methods.

Statistical Analysis

The percent coefficient of variation (%CV) and standard deviation (SD) were calculated for each paired bilateral arm measurements as follows:

$SD = (d^2/2)^{0.5}$ and $\%CV = (SD/M) \times 100$

where d denotes the difference between the two measurements and M denotes the mean. From these calculations the precision error also called technical error of measurement (TEM) was derived from the following expressions

$RMS\text{-}SD = (\Sigma SD^2/N)^{0.5}$ and $RMS\text{-}\%CV = (\Sigma CV^2/N)^{0.5}$

where RMS is the root mean square of the paired measurements and N is the number of patients.

The least significant change (LSC) is the smallest change between 2 measurements taken over time that must be exceeded for the change to be considered true with 95% confidence. This is expressed as 2.77 times precision; 2.77 x RMS-SD and 2.77 x RMS-%CV. Theoretically, it is limited to the device from which the precision was determined as the precision may vary with different devices (Shepherd JA, 2007).

Paired t-tests were used to compare differences in variables (e.g., fat mass, lean mass, ECF, bone mineral content) between the lymphademetous (LE) and non-affected (NA) arms.

Results

Table 4 details the differences between the measurements of the LE and NA arms for the 24 women obtained from DXA and BIS. The LE arm was significantly ($p < 0.0001$) greater than the NA arm for all the variables except the bone mineral content. The measured percent differences between LE and NA arms in this cohort of women with Stage 2 lymphedema ranged from 15% to 22.6%. To be noted is that the total arm volume derived from the fat, lean and bone mass DXA measurements and the CM of total volume yielded a similar percentage difference

between LE and NA arms. From the DXA measurements we found the FM of the LE arm was greater than the NA arm with a mean difference of 15.7% while the FFM of the LE arm was greater than the NA arm with a mean difference of 15.6%. As a point of reference, a recent retrospective study, using DXA to measure, involving 1240 healthy women ranging in age from 20-80 found the FFM of the right arm was higher than the left with a mean difference of 5% in all age groups yet only a slight difference of FM between the left and right arms of women over 50 years of age (Coin A, 2012). Table 4 also shows a change in the relation between LE and NA arms after their ECF volumes, as derived from the BIS, are subtracted from their lean mass volumes. There is a reduction in the percent difference from 15.6% to 8.7% between LE and NA arms for the FFM; this percent difference is still significant.

The precision of the various measurements are listed in Table 5. Precision of DXA mass measurement for the LE arm varied from 1.28% CV (BMC) to 1.49% CV (lean mass) and for the NA arm the precision ranged from 0.95% CV (lean mass) to 1.72% (BMC). The precision of BIS measurements for LE arm were 1.65 % (ECF) and 1.86% (total fluid volume). For the NA arm BIS precision values were 1.51% (ECF) and 1.56% (total volume). Precision for the anatomic tape measurement of volume was 1.71% CV for LE arm and 2.51% CV for NA arm. From these determinations LSC at a 95% confidence level was calculated (Table 7). With respect to DXA fat mass, for example, a measured change ≥ 57.3g or a change in %CV ≥3.91 in the LE arm would indicate, with a 95% confidence level, a significant change in fat mass. The calculations of LSC at a 95% confidence level for BIS measures of LE arm were 43.7ml, 4.57%CV (ECF) and 90.3ml, 5.15% CV (total fluid).

Discussion

The findings of this study show that DXA and BIS can be used to determine differences in upper limb tissue composition and upper limb volume. We found no significant difference in precision of the DXA or BIS measurements between LE arms and NA arms. Our results show the total volume derived from DXA measurements and the CM total volume measurements yielded similar percentage differences between LE and NA arms. The discrepancy in the actual volume is attributed to the fact that there were different tissue volumes being measured in all three methods. For instance, the DXA field extended from the finger tips to the gleno-humeral joint, while the circumferential volume limits were from the wrist to several centimeters below the gleno-humeral joint and, the BIS measured from the wrist to an indeterminate point between the axilla and acromion. Importantly, although all three methods are not measuring the exact same volume, this should not affect precision.

A relatively poor inter-subject (e.g., the arm position changing the shape of the arm) and inter-tester (e.g., the degree of tightness with which the tape is applied) repeatability can influence the precision error of the measurements derived from the circumferential tape technique. Furthermore, the truncated cone method that is typically used to calculate arm volume includes the inaccurate assumption that limb segments are cone shaped and does not allow for uneven skin surface. In this study, the precision results for the anatomic tape measurement of volume were 1.71% CV for LE arm and 2.51% CV for NA arm. These coefficients of variation rank the CM measurements as the least precise of the three methods analyzed in this study.

From a research or clinical perspective, with total volume as the only result obtainable from CM, this evaluation method has limited utility for the assessment or tracking of body composition in later stage BCRL. In contrast, the DXA measures give a breakdown of the tissue differences between limbs, thereby enabling the tracking of differences in fat and lean volume over time.

Clinically, the values for the LSC at a 95% confidence level calculated from the measured precision errors should serve as a guide to determine a significant change between two points in time such as when tracking the progression of this chronic condition. For example, as previously stated, a DXA measured change ≥ 57.3g of FM and/or BIS measured change ≥ 43.7ml of ECF within the LE arm would indicate a significant change that may require a specific treatment or change in treatment plan. Lesser degrees of confidence enable smaller changes to be considered normal; e.g., the LSC for fat at a 95% level of confidence for the LE arm is 2.77 x 1.41% = 3.9%, whereas for an 85% confidence the LSC is 2 x 1.41% = 2.8% (Table 5).

It is well known caveat that different manufacturers, instrument models, and software versions as well as different operators are all likely to affect the precision and reproducibility of the DXA results (Guglielmi1 G, 2012), (Toombs RJ, 2012), (Baim S, 2005). It is important to note that the lean mass as measured by DXA includes not only muscle, but all the other tissue components except fat and bone mineral (e.g. water, proteins, glycogen, non-bone minerals etc.). There is a report wherein the DXA lean mass difference between LE arm and NA arm was attributed entirely to muscle without correcting for the presence of ECF (Brorson H O. K., 2009). Our finding of a change in the relation between LE arm and NA arm after the ECFs are subtracted from their respective lean mass volumes show a reduction in the percent difference between LE arm and NA arm lean mass from 15.6% to 8.7%. The difference is still significant and may be due primarily to a difference in muscle mass. The BIS measurements of ECF indicates that a considerable portion of that lean mass difference found in the DXA measurements can be attributed to the fluid component of the lymphedematous arms.

Even though perometry and circumferential tape measurements are still the most commonly used clinical assessment techniques to identify the appearance of LE, other more sensitive and

reliable methods have been gaining recognition (Czerniec S. A, 2010),(Maher, 2012), (Cornish, et al. 2001). Over ten years ago, Cornish et al (2001) demonstrated the efficacy of BIS over CM in terms of its sensitivity and specificity in being a reliable measurement tool to determine the early detection of LE in 20 women up to 24 months following post-surgical procedures for breast cancer (Cornish, et al., 2001). More recently, BIS has been found to be more sensitive than perometry in determining more localized LE (Czerniec S.A, 2010). In fact, the lower level of detection of LE utilizing BIS has been shown to be approximately 35ml (Maher, 2012); a value that is very similar to the least significance difference (43.7mls) found in the present study. Taking these findings into account, it would appear that the BIS technique is sensitive, reliable, and has the precision to detect the onset of fluid change that would signal the early development of LE. However, for those women who progress through the various stages of LE, there may be a greater need and use for detection devices such as the DXA that we have found to be sensitive to soft tissue changes (e.g., adipose tissue) in this particular population.

The calculations used to estimate fluid volumes with the impedance and resistance data collected with the BIS incorporates the assumption of constant coefficients of resistivity for the fluids. These coefficients of resistivity were derived from a cohort of women without lymphedema. Most likely as a result of the protein rich ECF and other resistive sensitive material (e.g., fat deposits) found in lymphedematous limbs, the coefficient of resistivity would not be the same as that for non-affected arms and thus may be a source of error in the fluid volume calculations for the lymphedematous arms derived from the BIS. More research appears warranted to determine to what extent the body composition of the arms of women with BCRL varies over time. Although our findings have shown that the BIS measurements are less precise than DXA, we wish to point out the fact that the difference in precision between the two instruments is relatively small. This should not detract from using the BIS since, unlike the DXA

scanner, tracking patients with the BIS gives information about the fluid in the limbs initially and over time. As well the BIS is portable making bedside assessments possible; the time involved in measurement is about half that of a DXA scan and the BIS system is a fraction of the cost of the DXA scanner. The clinical need for the DXA measurements lie in those cases of BCRL where the difference in fluid volume do not account for the better part of the difference in total limb volume. As well tracking patients using DXA measurements would open a window to observing if the fat content responds to traditional decongestive therapy. Hence, performing assessments of BCRL with DXA and BIS in concert will yield a more complete picture of the tissue composition and may influence treatment management.

All the technical procedures in this study were conducted by one person and although this may be ideal, it does not reflect the clinical reality wherein the patient may be monitored with these procedures over time by different personnel. The inter-individual variation in precision was not an end point in this investigation.

In conclusion, DXA and BIS are two tools with good precision for research and clinical assessment of breast cancer related lymphedema. This finding is essential for us to acquire greater insight into the changes in tissue composition occurring with the progression of BCRL and for the development of novel treatments for this chronic condition.

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University Health Center's Research Ethics Board. This research was carried out in accordance with the Canadian government's Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans and the Helsinki Declaration of 1975, as revised in 2008.

Table 4: Dual energy x-ray absorptiometry, multifrequency bioelectric impedance spectroscopy and circumferential tape measurement of arm volumes in women (n=24) with Stage II breast cancer related lymphedema

Variable	Lymphedema arm	Non-affected arm	P	% difference
DXA, fat mass (g)	1409 (298)	1214 (270)	<0.001	15.7
DXA, lean mass (g)	2100 (390)	1813 (177)	<0.001	15.6
DXA, BMC (g)	141 (17)	140 (17)	0.832	0.6
DXA, total arm volume (ml)	3531 (574)	3070 (385)	<0.001	15.0
BIS, EFC (ml)	927 (165)	756 (89)	<0.001	22.6
BIS, total fluid volume (ml)	1806 (342)	1517 (163)	<0.001	19.0
Circumferential arm volume (ml)	2432 (386)	2135 (304)	<0.001	13.8
Lean mass volume minus ECF(ml)	1010 (161)	929 (130)	0.048	8.7

All values are expressed as mean ± standard deviation (SD). BMC: bone mineral content; DXA: dual energy x-ray absorptiometry; EFC: extracellular fluid volume; MF-BIS: multifrequency bioelectric impedance spectroscopy

Table 5: Precision expressed as root mean square (RMS) of the variables in the lymphedematous and non-affected arms

Variable	Lymphedema arm		Non-affected arm	
	RMS-SD (LSC)	RMS-%CV (LSC)	RMS-SD gm (LSC)	RMS-%CV (LSC)
DXA fat mass(g)	20.7 (57.3)	1.41 (3.9)	12.4 (34.3)	1.05 (2.9)
DXA lean mass (g)	30.6 (84.7)	1.49 (4.3)	17.4 (48.2)	0.95 (2.6)
DXA bone mineral content (g)	1.8 (5.0)	1.28 (2.8)	2.5 (6.9)	1.72 (4.8)
DXA arm volume (ml)	42.0 (116)	1.16 (3.2)	18.3 (50.7)	0.62 (1.7)
BIS extracellular fluid (ml)	15.8 (43.7)	1.65 (4.6)	11.5 (31.8)	1.51 (4.2)
BIS total fluid (ml)	32.6 (90.3)	1.86 (5.2)	23.2 (64.3)	1.56 (4.3)
Circumferential arm volume (ml)	44.8 (124)	1.71 (4.7)	52.3 (145)	2.51 (6.9)

LSC: least significant change for 95% confidence

Manuscript 2

A comparison of three intervention methods for women with breast cancer-related lymphedema: a pilot study

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ABSTRACT

The incidence of breast cancer-related lymphedema (BCRL) is approximately 30% for women who undergo breast cancer treatment (e.g. mastectomy, axillary node dissection and/or radiation treatment) (Williams A, 2005). The resulting physical symptoms (e.g., aching, numbness, pain and fatigue), functional outcomes (e.g., decreased strength and range of motion), and the need to wear compressive garments on a daily basis for prolonged periods of time place a persistent and significant burden on the quality of life of these survivors and creates the need for effective treatment interventions. Although there are a growing number of different treatment interventions, little is known about the efficacy of these treatments in terms of either maintaining or reducing BCRL. Therefore, the study objectives were: 1) To assess the immediate and short-term effects in extracellular fluid (ECF) following a single bout of activity from three different interventions for BCRL (e.g., manual lymphatic drainage (MLD), aqua lymphatic therapy (ALT), and a Casley-Smith based exercise routine (CSER), 2) To determine which of the three interventions is most effective in maintaining or reducing limb volume over a 24 hour period, 3) To identify any changes in physical symptoms after each intervention and, 4) To ascertain which of the three interventions the women prefer. **Methodology**: The participants were sixteen women between the ages of 35 and 75 with Stage II lymphedema. All participants were required to refrain from wearing their compression garments during interventions as well as 24 hrs before and after each intervention. Each of the 3 interventions was completed once and lasted approximately 45min. **Procedures:** Bilateral limb measurements to estimate changes in limb volume were obtained using bio-impedance spectroscopy (BIS) and circumferential arm measures (CAM). The Lymphedema Breast Cancer Questionnaire (LBCQ) was administered to subjectively gauge any change in symptoms. After completion of the final intervention, a questionnaire on intervention preference was administered. **Results:** There were

no significant increases in ECF or CAM following a single bout of each intervention over the 24 hr recovery period. Of the three interventions, **ALT** was the only intervention that showed a significant ($p \leq 0.05$) decrease in ECF from baseline to 20-24hrs post-intervention. When compared to the MLD, the ALT intervention demonstrated a consistent 3.31% greater ($p=0.038$) reduction in ECF volume over 24hrs. Comparing physical symptoms experienced by the participants in each of the three interventions, there was no significant differences found pre and post interventions. The majority of women (13/16) preferred ALT (7/16) and MLD (6/13).

Conclusion: In the immediate to short-term post-intervention period, these preliminary findings show that none of the three interventions increased ECF and physical symptoms in women who had removed their compressive garments Thus, ALT and CSER appear to be safe exercise interventions in women with BCRL; however, ALT was the most effective intervention in reducing limb volume over short term (24 hrs) post-intervention period.

Abbreviations

ALT	Aqua Lymphatic Therapy	DXA	dual energy x-ray absorptiometry
BCRL	breast cancer related lymphedema	ECF	extracellular fluid
BIS	bioelectric impedance spectroscopy	LBCQ	Lymphedema Breast Cancer Questionnaire
CAM	circumferential arm measurements	LE	Lymphedema
CDT	Complete (or Complex) Decongestive Therapy	NA	non-affected
CSER	Casley-Smith based exercise routine	MLD	Manual Lymphatic Drainage

INTRODUCTION

Breast cancer-related lymphedema (BCRL) is an incurable condition that results in swelling of the arm(s), hand(s) and /or breast caused initially by an accumulation of protein-rich interstitial fluid with the possibility of the long-term build-up of subcutaneous fat deposits and fibrous matrices. This fluid increase could result from the treatment of breast cancer, such as surgical intervention (e.g. radical mastectomy and axillary node dissection) and/or radiation treatment. Velanovich & Szymanski (1999) revealed that for a significant number of patients with BCRL the disorder is physically as well as emotionally disabling. The adverse physical effects (e.g., stiffness, numbness, and fatigue) and negative functional outcomes (e.g., decreased strength and range of motion) place a constant and significant burden on women with BCRL that worsens their quality of life (Passik, 1998).

According to the Position Statement of the National Lymphedema Network, the most appropriate course of treatment upon diagnosis of BCRL is Complete (or Complex) Decongestive Therapy (CDT) which consists of two phases (Network, 2009). Phase I focuses on volume reduction of the lymphedematous limb and attending to any skin irritations and/or infections which are common sequelae to impaired lymphatic function. This phase comprises an intensive 2-6 week course utilizing four treatment methods: 1) manual lymphatic drainage (MLD); 2) skin care; 3) daily compression bandaging and 3) remedial exercises. This phase continues until the limb volume reduction reaches a steady state plateau at which time the patient moves on to Phase II. Phase II of the CDT protocol is considered to be a maintenance period. The patient is responsible for Phase II and will need to take self-directed measures for the rest of their lives to maintain the limb volume reduction that was achieved in Phase I. Standard practice for clinicians treating BCRL is to educate the patient throughout Phase II on

skin care, self massage and home-based exercises (Network, 2009). It is believed that the absence of any self-directed measure to control LE and its symptoms would typically result in the worsening of the BCRL condition (Bar Ad, et al., 2010), (Johnstone, 2006). Despite the education and advice given to patients diagnosed with BCRL concerning the importance of the maintenance phase of BCRL treatment, there is evidence to show that some patients have difficulty adhering to the Phase II program resulting in the progression of lymphedema to levels observed prior to CDT (Vignes, 2011). One of the complications that some patients encounter during their maintenance program is the adverse effects of wearing compressive garments. For instance, compression of the arm garments worn at the wrist can cause hand swelling, and friction from the seams of the garment can cause irritation of the skin and pain (Vignes, 2011).

In Quebec, the cost of lymphedema treatments is not covered by Medicare. According to a recent publication on the Quebec government website the cost of compressive garments alone is $1,166 per person per year ((INESS), 2011). This does not include the cost of treatment interventions. The cost of one MLD (skin massage) treatment ranges between $80- $100. Since LE in most cases is a chronic lifelong condition, the accumulated expenses for regular treatments clearly place a serious economic burden on the patient. Although MLD has been shown to enhance the effects of compression treatments (Lasinski B, 2012), this treatment has been found to be ineffective in maintaining limb volume over the long term (Vignes, 2011). For those with BCRL who have limited financial resources or intolerance to compressive sleeves their lifelong, self-care program may be highly dependent upon self-massage and remedial exercises as a means of maintaining their limb volume. Consequently, there should be a particular importance and emphasis on the types of interventions available to these women.

Using BIS and CM to assess changes in limb volume and self-reported symptoms as outcome measures, this study investigates the short-term efficacy of three interventions for the treatment of BCRL: 1) Manual Lymphatic Drainage (MLD), 2) Aqua Lymphatic Therapy (ALT) and 3) Casley-Smith based Exercise Routine (CSER).

METHODS

Experimental Design**:** A repeated measures crossover design ("3 treatments, 4 periods") was chosen to reduce the chance of variation between individuals that may skew the results and also because this design is most suited for the comparison of smaller groups.

Participants: Recruit of the study population took place at the McGill University Health Center (MUHC) Lymphedema Clinic between September 2011 and May 2012. Nineteen women who were diagnosed with Stage II unilateral BCRL were recruited from the 35 eligible participants visiting the Clinic. Written informed consent was obtained from all participants. Eligibility criteria for the study included: 1) females between 35-75 years of age, 2) BMI ≤ 45, 3) six months or more post-breast cancer treatment, 4) breast cancer Stages I-III in remission, 5) absence of any significant chronic cardiovascular, metabolic, and neurological diseases, 6) the presence of unilateral Stage II lymphedema, 7) not pregnant, 8) no aversion to swimming pool activities, 9) completion of Phase I of CDT, 10) willingness to refrain from wearing a compression garment for 24 hours prior to and following each intervention. Lymphedema staging was determined according to the International Society of Lymphology criteria (Lymphology, 2009).The staging assessments were performed by the clinic's physician and physiotherapists who are lymphedema specialists.

Of the 19 women recruited, three withdrew (two found time commitment too great, one experienced minor shoulder discomfort after the CSER intervention and was treated by a

physiotherapist) resulting in 16 women completing the protocol. See Table 6 for a description of the participant characteristics.

Protocol

The assessments involved the LBCQ symptom questionnaire, bilateral limb volume measurements for ECF using bioelectrical impedance spectroscopy (BIS) and circumferential arm measures (CM). The unaffected limb was measured to serve as an internal control. Participants were assessed before and after each treatment intervention. For this repeated measures crossover design, all women participated in each of the three treatment interventions over a five -seven week period, with a 10 day minimum “washout” interval in between interventions. All participants were asked to refrain from wearing a compression garment during the intervention as well as 24 hours prior to and following each intervention (for a total of 48 consecutive hours). The MLD and CSER interventions took place at the McGill Nutrition and Performance Laboratory of the McGill University Health Centre (MUHC). The ALT intervention took place in the swimming pool at the Montreal YM-YWHA and was performed by a physiotherapist from the MUHC. The MLD sessions were performed by a certified Vodder method trained therapist. All interventions were performed by trained lymphedema specialists and each session lasted for 45 minutes. All measurements using the BIS device and the CAM tape measure were performed by one of the authors (AN) who had had three years of experience in the use of both measurement methods.

Interventions

ALT is a self-treatment in a group setting. ALT is based on the Casley-Smith remedial exercise principles. The method uses the anatomical principles of the lymphatic system and the properties of water to achieve the goals of lymphedema therapy. The water temperature ranges

from 31°C to 33°C. Water's property of buoyancy aids in elevating the limbs and thus allowing for the performance of exercises and self massage with minimal effort (Tidhar D., 2010). The possible mechanism behind this treatment is twofold: 1) incorporating water's hydrostatic property to create a pressure gradient enhancing directional lymphatic flow, and 2) the viscosity of water providing resistance with movement for a gentle massage or more vigorous movements in specific directions for strengthening and encouraging the extrinsic pumping of the lymphatic vessels.

The MLD method of massage stretches the skin and incorporates gentle rhythmic circular movements in specific directions, interspersed with rest periods, using an approximate pressure of 30mmHg which varies according to the edema (e.g., increased pressure for breakdown of fibrotic tissue). The suggested mechanism of this intervention to encourage lymph flow is threefold: 1) shunting the lymph with slowly increasing pressure, 2) an attempt to create pumping by the rhythmic pressure, rest (no pressure) movements, and 3) an attempt to stimulate lymphatic vessel contraction with the stretching of the skin (Williams A., 2010), (Kasseroller, 1998).

CSER is a gentle (low intensity) 45 minute exercise routine that incorporates breathing, slow rhythmic mild isotonic contractions of the arm muscles interspersed with self-massage to promote regional lymphatic fluid movement and venous outflow (Casley-Smith J.R, 1998). The mechanism for this method is proposed to be the systematic application of varying pressures through movements to specific areas with the intent to create a pressure gradient to encourage the directional flow through the lymphatic pathways.

Outcome measures

Anthropometry: Before each intervention session body weight was measured to the nearest 0.1 kg using a digital scale (Amcells TBS Series, USA). Height was measured without shoes to the nearest 0.5cm using a mechanical stadiometer (Seca). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared (kg/m^2).

To minimize a possible order effect, participants were assigned to receive the three interventions in one of five sequences. The pre-intervention symptom questionnaire was administered and bilateral limb volume measurements were taken immediately prior to each intervention. The post-intervention assessments included a 12 question abridged version of the LBCQ and were done at 10min, 60min and 20-24 hrs.

Bilateral changes in limb volume were measured using the following two methods:

1) Bioelectrical impedance spectroscopy (BIS): This method has been shown to be highly reliable with good precision for the assessment of BCRL (Cornish, et al., 2001), (Newman A, 2013). The BIS system used in this protocol was the ImpediMed SFB7. For the study analysis, the system's software (Bioimp version 2.25, ImpediMed Ltd., Brisbane, Australia) was used with the parameters set as follows: a data rejection level of "none" (default setting) and an operator-chosen frequency range of 3 kHz-500 kHz. These settings were previously determined to be the most appropriate for this population (Newman A, 2013). Participants were in a supine position on a non-conductive table with their arms slightly abducted, palms down, and with legs slightly apart. The skin was wiped with an alcohol swab and allowed to dry. Four electrodes were applied to the skin in accordance with manufacturer's recommendations. With four leads attached to the electrodes, a small constant AC current (200μA) was passed from hand to foot and the drop in voltage was measured. The measures of impedance (opposition to the flow) of

the electric current through the body fluids are used to calculate the volume of extracellular and intracellular fluid (Ward, Czerniec, & Kilbreath, 2009).

2. Circumferential arm measures (CM): This method makes use of a tape measure to obtain circumferential measures from the wrist to axilla at 10-cm intervals. Arm volumes were calculated by summing the various segment volumes derived from the truncated cone formula (Karges, 2003). CAM is a commonly used method for monitoring limb volume in the treatment of lymphedema and has been found to be highly reliable and correlates well with water displacement that is presently recognized as the clinical 'gold standard' method for limb volume measurement (Gjorup C, 2010), (Czerniec S. A, 2010), (Maher, 2012).The assessor was blinded to previous measurements by using a new data collection sheet for each set of measures. A more detailed description of the measurement protocols can be found in our previously published work (Newman A, 2013).

These assessment periods lasted 10-20minutes and were done pretreatment and at 10min, 60min and 20-24 hrs following each treatment intervention.

Data Analysis

The statistical package used was NCSS Statistical Software (version 7.1.17 Kaysville, Utah). The analysis done was a 3 (interventions) x 4 (time periods) repeated measures analysis to estimate the variance among the volume measurements for each of the three treatment interventions. A $p \leq 0.05$ was used to determine if the variance estimate was considered to be statistically significant. When a p-value of ≤ 0.05 was obtained, the Tukey-Kramer Multiple-Comparison post-hoc test was run to determine significant differences between individual means. The quantitative analysis of the LBCQ questionnaires was performed using a χ^2 (Chi squared) test.

RESULTS

Within group differences: There were no significant changes from baseline ECF at any of the measured time points (10 min, 60min, 24 hrs) following MLD or CSER. Of the three interventions, only ALT had a statistically significant ($p \leq 0.05$) change from baseline in ECF volume (26.9mls) at the 24-hour post-intervention measurement period (Table 7).

Between group differences: In comparing the ALT vs MLD interventions from baseline to 20-24hrs, ALT has a 3.3% greater ($p=0.038$) mean percentage ECF volume change than MLD at 20-24 hours (Table 8). No other ECF differences exist between ALT vs CSER or MLD vs CSER. With respect to circumferential arm measurements, there was a significant difference ($p=0.021$) in the percentage change between ALT and MLD at 10 min post intervention. No other circumferential arm measurement differences exist between ALT vs CSER or MLD vs CSER.

There was a significant ($p \leq 0.05$) main effect between ALT and MLD. Post-hoc analyses revealed differences in ECF with significantly greater volumes for ALT than MLD at baseline ($p=0.016$), 10 minutes ($p=0.022$) and 60 minutes ($p=0.009$) post-intervention (Table 9). Mean ECF volume for CSER was significantly less than ALT at 10minutes ($p=0.004$) and significantly lower than MLD at 60 minutes ($p=0.004$) see Table 9.

Abridged LBCQ questionnaire and subjective preferences

The results of the abridged LBCQ questionnaire of symptoms showed that for symptoms experienced during assessments, there were no significant differences between groups. The two most common symptoms reported in all three intervention sessions were swelling and heaviness. There was no clear consensus on the most preferred choice of intervention;

however, the majority of participants preferred ALT (7/16) and MLD (6/16). The treatment that participants thought to be the most effective subjectively was ALT (10/16).

DISCUSSION

There were two main objectives to this study. First, we wanted to determine if there would be a significant increase in ECF measured by BIS and limb volume by CM following a single bout of each intervention treatment in women who have had their compression garments removed for 48 hrs. Secondly, we wanted to determine which of the three interventions if any, is most effective in reducing limb volume in the immediate (10-60 min) and short term (24-hour) post-intervention time period. The results of this study suggest would tend to suggest that the ALT intervention was the most effective of the three interventions in terms of significantly reducing ECF volume not only over time but also in comparison to the MLD intervention. In an earlier study involving ALT, Tidhar et al. (2010) had a mean volume reduction of 53.5ml measured with water displacement immediately following the first ALT session. With respect to MLD, post-intervention limb volume did not change. Although it is tempting to state that MLD maintained limb volume constant over time; however, without the comparison of a control group, we cannot equivocally state that MLD had any effect whatsoever. However, in a study by Maher et al. (2012), MLD with the compressive sleeve was compared to MLD alone found there was no change in volume for either intervention at 30 min intervals for 2 hrs using BIS and perometry. Similarly, CSER did not produce any ECF volume change at any of the post-intervention time points. Moseley et al. (2005) completed a brief 10 minute gentle arm exercise with deep breathing and found an immediate median reduction of arm volume of 52mls ($p=0.004$) that was not sustained at the 60min mark when the median volume returned to baseline. Taking into account the three treatments, it is encouraging from a clinical perspective to observe that ECF

did not increase in the short- term post intervention period. Whether repeated sessions of ALT or CSER will have a greater impact on the limb volume of persons with BCRL over the longer term remains to be seen.

Limitations: As mentioned previously, there was no control group included in the design of this pilot study. Since this was designed to be a pilot study, we knew that the sample size used would underpower the study and prevent us from making any significant generalizations about our findings. In fact, one of the secondary hypothesis to this pilot study was to determine what sample size would be needed in order to adequately power the follow up study. By calculating standardized difference = target difference / standard deviation and referring to a nomogram produced by Altman (Altman, 1991), we are able to determine that it would require 50 participants to reach a suitable level of power (0.8).

Normally, we would not have expected the baseline ECF volume measurements to be any different from one another prior to each intervention. However, this was not the case for the baseline ECF measurement prior to ALT which was statistically higher that the baseline MLD value. In a previous study involving ALT, Tidhar et al. (2010) found that there was no correlation between initial arm edema and the reduction in the volume after the ALT intervention (linear correlation of 0.3, $p>0.2$). Perhaps the difference in baseline volume prior to ALT was related to the women refraining from wearing their compression garment; however, this seems unlikely since we did not see any similar increases in baseline BIS prior to the other interventions. Another limitation to the research design was in not measuring the arm volume of the participants immediately after removing their compressive sleeves. Since the baseline measurements for ALT were done in a public pool setting, it is reasonable to assume that

perhaps the hot humid environment of the public pool had a vasodilatory impact on the lymphedematous arm thereby increasing the volume of the ECF compartment.

The time commitment for participants in this study was approximately 12 hours over a minimum of 5 weeks. Several potential participants declined to participate indicating they found the time commitment too onerous. This factor along with the physical activity requirement to fulfill the protocol may have deterred less motivated and/or less physically active LE patients from participating and therefore an overall response bias may exist. Another limitation of this study is the lack of controls for participants' daily activities during the 24 hour period immediately following the intervention before the final assessment. The activities of the 16 women who participated in this study, during the 24hr period between assessments varied from staying at home resting, to assisting family in the hospital, to a day of physical work.

Recommendations for future research

This study has set the stage for future studies on the topic of remedial exercise interventions in women with Stage II lymphedema. We have been able to determine what sample size is needed for a larger and better powered investigation. Since the present study focused on the immediate short-term effects of each intervention, we now need to track potential ECF volume changes over longer periods for ALT and CSER with and without compressive garments. A longitudinal study design by Vignes et al. (2010) could serve as a model whereby we will examine the long- term post intervention changes following ALT and CSER with and without wearing a compressive sleeve.

CONCLUSION

For women with Stage II BCRL, ALT and CSER interventions as well as MLD appear to be safe, show no increase in ECF or physical symptoms, despite the fact that these women had removed their compression sleeve for 48 hrs. Although ALT appears to be the most effective intervention in terms of reducing limb volume over a 24 hour period, this finding should be viewed with some caution since this change appears to be mediated by yet an unexplained elevated baseline BIS value. Clearly demonstrating the existence of affordable intervention(s) that are effective in either reducing or at least maintaining the volume of their lymphademetous limb may assist women with BCRL with their lifelong challenges.

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Approval for this study was obtained from the McGill University Health Center's Research Ethics Board. This research was carried out in accordance with the Canadian government's Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans and the Helsinki Declaration of 1975, as revised in 2008

Table 6: Participant Characteristics n=16

Age (SD)	60.23 (7.21)
Weight (kg) (SD)	74.9 (15.04)
BMI (kg/m²) (SD)	29.62 (4.86)
Time since BCa Tx (months) (SD)	102.7(82.80)
Time since LE Dx (months)(SD)	78.8(73.40)
Breast cancer treatment	
Radiotherapy (n)	16
Chemotherapy (n)	15
ALND only (n)	3
Lumpectomy (n)	7
Mastectomy (n)	6

All values are expressed as mean ± standard deviation (SD);
ALND: Axillary lymph node dissection; BMI: Body mass index;
BCa Tx: breast cancer treatment; LE Dx: lymphedema diagnosis

Table 7: Effects of three treatment interventions on absolute changes in ECF volume (mls)

	Pre-treatment	Post-treatment		
Treatment	0 minutes	10minutes	60 minutes	20-24hours
ALT	883 (188)	881 (202)	873 (198)	856 (200)*
MLD	856 (189)	855 (180)	845 (180)	868 (201)
CSER	858 (202)	845 (192)	849 (198)	858 (213)

All values are expressed as mean ± standard deviation (SD); N=16/treatment; *: significant change from baseline (≤ 0.05); **ALT***: Aqua Lymphatic Therapy;* ***CSER****: Casley-Smith Exercise Routine;* ***MLD:*** *Manual Lymphatic Drainage*

Table 8: BIS (ECF) Difference of the means between treatments as a function of time

N=16	Time (min)	MLD (ml)	CSER (ml)
ALT	0	27.8 (0.016)*	15.8 (0.215)
ALT	10	32.3 (0.022)*	31.2 (0.004)*
ALT	60	34.7 (0.009)*	15.7 (0.607)
ALT	1200	9.6 (0.396)	9.6 (0.445)
MLD	0	----------	11.9 (0.357)
MLD	10	----------	1.06 (0.912)
MLD	60	----------	18.9 (0.004)*

All values are expressed as mean ± standard deviation (SD);
* denotes significant difference ($p \leq 0.05$) from baseline;
ALT: Aqua Lymphatic Therapy; **CSER**: Casley-Smith Exercise Routine;
ECF: extracellular fluid; **MLD**: Manual Lymphatic Drainage

Table 9: A comparison of treatment interventions measured in percentage changes from baseline ECF over time using bioelectric impedance spectroscopy

	Pre treatment	**Post-treatment**		
Treatment Comparison	Time (0 minutes)	10 minutes	60 minutes	20-24 hours
ALT vs MLD	0	0.208 (0.876)	-0.823 (0.394)	-3.308 (0.038)*
ALT vs CSER	0	1.475 (0.201)	-0.989 (0.302)	-2.861 (0.069)
MLD vs CSER	0	1.266 (0.091)	-0.166 (0.867)	0.494 (0.818)

All values are expressed as mean ± standard deviation (SD); * denotes significant difference ($p \leq 0.05$) from baseline; **ALT**: Aqua Lymphatic Therapy; **CSER**: Casley-Smith Exercise Routine; **ECF**: extracellular fluid; **MLD**: Manual Lymphatic Drainage

Table 10: A comparison of treatment interventions measured in percentage changes from baseline circumferential arm measurements

N=16	**Time (min)**	**MLD-CMvol (percentage points)**	**CSER- CMvol (percentage points)**
ALT- CMvol	0	0	0
ALT- CMvol	10	1.868 (0.021)*	-0.496 (0.577)
ALT- CMvol	60	-1.186 (0.211)	-1.826 (0.082)
ALT- CMvol	1200-1440	-0.509 (0.549)	-0.093 (0.914)
MLD- CMvol	0	----------	0
MLD- CMvol	10	----------	-0.669 (0.354)
MLD- CMvol	60	----------	-0.640 (0.458)
MLD- CMvol	1200-1440	----------	0.415 (0.555)

All values are expressed as mean ± standard deviation (SD); * denotes a significant ($p \leq 0.05$) difference; **ALT**: Aqua Lymphatic Therapy; **CMvol:** Circumferential measures, limb volume**; CSER**: Casley-Smith Exercise Routine; **MLD:** Manual Lymphatic Drainage

CONCLUSIONS

These findings suggest that ALT was the most effective intervention in reducing limb volume over a 24 hour period. However, in light of the small sample size and the results of significantly greater ECF volume for the ALT group at baseline, there is reason to question the results rather than draw any conclusions. There is a definite need for research studies similar to this one with a larger sample size and more controls. Improvements to the research design of this study would include a sample size of 50 or more participants a control group each wearing a compressive sleeve, tracking the activities of the participants over the 48 hours that the participants were not wearing their compressive sleeves, a training session for the two exercise interventions, and measurements pre and post ALT to be taken in an environment similar to the environment where the other two interventions took place. Ideally, if feasible to measure again 48 hours post intervention to see if more would be revealed about the residual effect of each of the three interventions.

This study has set the stage for future studies on the topic of exercise interventions in women with Stage II lymphedema. We have been able to determine what sample size is needed for a larger and better powered investigation. Since the present study focused on the immediate short-term effects of each intervention, we now need to track potential ECF volume changes over longer periods for ALT MLD and CSER with and without compressive garments.

Women with BCRL live with many challenges. Clearly demonstrating that there exists inexpensive intervention(s) that are effective in reducing or maintaining the volume of their lymphademetous limb may assist them with their lifelong burden of treatment for their BCRL.

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APPENDIX A

Patient ID: _____ Date: _____

Pre-protocol Questionnaire

Which hand do you write with? _____

Which arm, hand is affected by lymphedema? _____

When was your lymphedema diagnosis? _____

When were you treated for breast cancer? _____

What type of treatment did you have for the breast cancer?

_____

_____

_____

APPENDIX B

Lymphedema and Breast Cancer Questionnaire (LBCQ)

ID Code #_____ Interview Date _____ Staff ID____

Answer all questions you understand. Do not answer questions that have any words you do not understand. Circle any words you do not know. Lymphedema means swelling of the arm, hand, shoulder or upper body on the side where your cancer was first treated. Now refers to today or in the past month.

Which side of your body was treated for breast cancer?

Left __ Right __ Both __

Questions about changes that have happened to your arm or body in the last month.

Have you had a change in arm size?

Larger __ Smaller __ No change __

Have you had a change in shoulder size?

Larger __ Smaller __ No change __

Have you had a change in neck size?

Larger __ Smaller __ No change __

Have you had a change in how your sleeve fits?

Looser __ Tighter __ No change __

Have you had a change in how your sleeve cuff fits?

Looser __Tighter __ No change __

Have you had a change in how your ring fits?

Looser __ Tighter __ No change __

The following questions pertain to your experiences with movement, use, and sleep now* and during the past year: * Now refers to today or in the past month.

Do you have limited movement of your...	Now If yes, please describe	During Past Year If yes, please describe.
8. Shoulder?	No __ Yes __	No __ Yes __
9. Elbow?	No __ Yes __	No __ Yes __
10. Wrist?	No __ Yes __	No __ Yes __
11. Fingers?	No __ Yes __	No __ Yes __
12. Does your arm or hand feel weak?	No __ Yes __	No __ Yes __
13. As part of your job, are you required to do some action with your arm or hand over and over?	No __ Yes __	No __ Yes __
14. Do you need pillows to support and raise your arm?	No __ Yes __	No __ Yes __
15. Are you unable to sleep through the night because your arm is not comfortable?	No __ Yes __	No __ Yes __

ID Code #_____ Interview Date _____ Staff ID____

The following questions pertain to arm, breast, and chest symptoms now* and during the past year: * Now refers to today or in the past month.

Have you experienced	**Now**	**During Past Year**	**What action you did for this symptom**: Please describe.
16. Tenderness?	No __ Yes __	No __ Yes __	No action __ Action:
17. Swelling?	No __ Yes __	No __ Yes __	No action __ Action:
18. Swelling with pitting*?	No __ Yes __	No __ Yes __	No action __ Action:
19. Redness?	No __ Yes __	No __ Yes __	No action __ Action:
20. Blistering?	No __ Yes __	No __ Yes __	No action __ Action:
21. Firmness/tightness?	No __ Yes __	No __ Yes __	No action __ Action:
22. Increased temperature in your arm?	No __ Yes __	No __ Yes __	No action __ Action:
23. Heaviness?	No __ Yes __	No __ Yes __	No action __ Action:
24. Numbness?	No __ Yes __	No __ Yes __	No action __ Action:
25. Stiffness?	No __ Yes __	No __ Yes __	No action __ Action:
26. Aching?	No __ Yes __	No __ Yes __	No action __ Action:
27. Chest wall swelling?	No __ Yes __	No __ Yes __	No action __ Action:

*Pitting: when you press firmly on your skin and the dent stays long enough to feel it when you slide the pad of your finger across it

ID Code #_____ Interview Date _____ Staff ID____

Have you experienced	**Now**	**During Past Year**	**What action you did for this symptom**: Please describe.
28. Breast swelling?	No __ Yes __	No __ Yes __	No action __ Action:
29. Pockets of fluid develop?	No __ Yes __	No __ Yes __	No action __ Action:
30. Other symptoms	No __ Yes __	No __ Yes __	No action __ Action:

31. What is your birthday? ____/ ____/ _____

32. What is the highest year of school you attended? _____

33. What is your height? _____

34. What is your weight? _____

35. How much did you weigh 6 months ago? _____

36. How much did you weigh 1 year ago? _____

37. Diagnosis with breast cancer_____ Date _____

38. Drugs (Chemotherapy) _____ Date _____

39. Surgery_____ Date _____

ID Code #_____ Interview Date _____ Staff ID____

40. Radiation_____ Date _____

41. Did you develop any complications related to your treatments?

Complication:	Date:	Treatment for Complication
_____	_____	_____

42. What other health problems do you have? _____

43. Have you had lymphedema before the breast cancer?

No __

Yes __

If yes, please describe.

44. Has anyone in your family ever had lymphedema?

No __

Yes __ If yes, please describe._____

45. Do you assist others with their daily care?

No __

Yes __

If yes, please describe. _____

ID Code #_____ Interview Date _____ Staff ID____

** Have you experienced swelling in the arm, hand, shoulder or upper body on the side where your cancer was first treated, or have you been diagnosed with lymphedema?

Yes__ No__ If YES, please answer questions 46-56. If NO, please go to question 57

The following questions pertain to changes in your life now and during the past year.

How has lymphedema changed your...	Now If yes, please describe.	During Past Year If yes, please describe.
**46. Mood?	No __ Yes __	No __ Yes __
**47. Lifestyle?	No __ Yes __	No __ Yes __
**48. Time?	No __ Yes __	No __ Yes __
**49. Finances?	No __ Yes __	No __ Yes __
**50. Body image?	No __ Yes __	No __ Yes __
**51. Relationship to primary care physician?	No __ Yes __	No __ Yes __
**52. Relationship to specialists?	No __ Yes __	No __ Yes __

**53. Do you believe your doctor has been interested in treatment for your lymphedema?

**54. Have you received helpful information for understanding and treating lymphedema?

**55. What do you think caused your lymphedema?

**56. What do you think makes it worse?

57. Do others assist you with your daily care?

No __
Yes __

If yes, please describe

1. If you have comments you would like to make about breast cancer and lymphedema, please use this space to share them with us.

APPENDIX C

Post-Protocol Questionnaire

Patient ID: _____ Date: _____

Which treatment intervention did you prefer, and why?

Manual Lymphatic Drainage (MLD) ____

Aqua Lymphatique Therapy (ALT) ____

Casley-Smith exercise routine (CSER) ____

_____

_____

Which treatment intervention seemed most effective?

MLD ____

ALT ____

CSER ____

Which treatment intervention would you most likely continue to use, and why?

MLD ____

ALT ____

CSER ____

_____

_____

_____

Patient ID:_____ **Appendix D** Date:_____

Lymphedema and Breast Cancer Questionnaire (LBCQ): modified version

	PRE	**POST**			
Are you experiencing:	**Time:**	**Time:**	**Time:**	**Time:**	**Time:**
12.Arm or hand weakness?	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __
16. Tenderness?	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __
17. Swelling?	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __
18. Swelling with pitting*?	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __
19. Redness?	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __
20. Blistering?	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __
21. Firmness/tightness?	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __
22. Increased temperature in your arm?	No __	No __	No __	No __	No __

	Yes __	Yes __	Yes __	Yes __	Yes __
23. Heaviness?	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __
24. Numbness?	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __
25. Stiffness?	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __
26. Aching?	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __	No __ Yes __

*Pitting: when you press firmly on your skin and the dent stays long enough to feel it when you slide the pad of your finger across it.

APPENDIX E

STATISTICS

Quantitative Analysis of the abridged Lymphedema Breast Cancer Questionnaire (LBCQ)

r × *c* Contingency Table: Results:

The results of a contingency table X^2 statistical test

data: contingency table

	A	B	C	
1	7	9	7	23
2	7	6	5	18
3	7	13	9	29
4	3	5	5	13
5	7	10	11	28
6	24	20	18	62
7	7	11	6	24
8	9	7	3	19
9	10	8	8	26
	81	89	72	242

expected: contingency table

	A	B	C
1	7.70	8.46	6.84
2	6.02	6.62	5.36
3	9.71	10.7	8.63
4	4.35	4.78	3.87
5	9.37	10.3	8.33
6	20.8	22.8	18.4
7	8.03	8.83	7.14
8	6.36	6.99	5.65
9	8.70	9.56	7.74

chi-square = 8.36

degrees of freedom = 16

probability = 0.937

APPENDIX E

Page/Date/Time 1 3/9/2013 10:37:00 PM
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Response **ECF_ALT**

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: PATIENT	17	325.0898	19.12293	2.61	0.005203*	
B: Time	3	113.3017	37.76723	5.16	0.003772*	0.900113
S	45	329.5029	7.322288			
Total (Adjusted)	65	772.1014				
Total	66					

* Term significant at alpha = 0.05

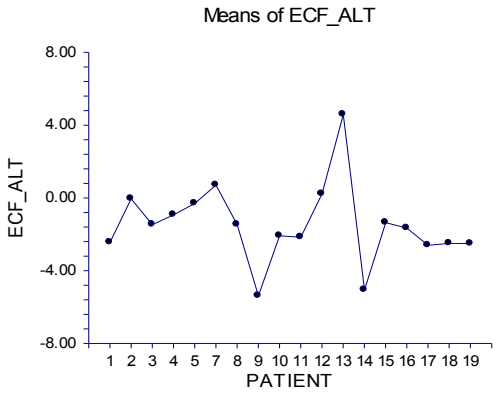
Means and Standard Error Section

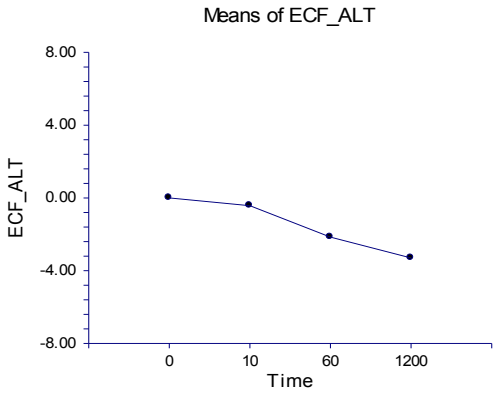
Term	Count	Mean	Standard Error
All	66	-1.472813	
A: PATIENT			
1	4	-2.45	1.352986
2	4	-0.05	1.352986
3	1	-1.472813	2.705973
4	4	-0.9325	1.352986
5	4	-0.305	1.352986
7	4	0.7025	1.352986
8	1	-1.472813	2.705973
9	4	-5.3825	1.352986
10	4	-2.0825	1.352986
11	4	-2.16	1.352986
12	4	0.215	1.352986
13	4	4.5775	1.352986
14	4	-5.0575	1.352986
15	4	-1.36	1.352986
16	4	-1.66	1.352986
17	4	-2.605	1.352986
18	4	-2.5	1.352986
19	4	-2.515	1.352986
B: Time			
0	18	-6.661338E-16	0.6378039
10	16	-0.429375	0.6764932
60	16	-2.1575	0.6764932
1200	16	-3.304375	0.6764932

Analysis of Variance Report

Page/Date/Time 2 3/9/2013 10:37:00 PM
Database D:\DOCUMENTS\CING SOUTH_FILE ... \DATA\MY DATA\3TREAT%ANNE.S0
Response ECF_ALT

Plots Section





Bonferroni (All-Pairwise) Multiple Comparison Test

Response: ECF_ALT
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=7.322288 Critical Value=2.7599

Group	Count	Mean	Different From Groups
1200	16	-3.304375	10, 0
60	16	-2.1575	
10	16	-0.429375	1200
0	18	-6.661338E-16	1200

Notes:
This section presents the results of all paired comparisons among the means. Since this procedure uses the Bonferroni inequality, it is not as accurate as the Tukey-Kramer's method.

Dunnett's Two-Sided Multiple-Comparison Test With Control

Response: ECF_ALT
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=7.322288 Critical Value=2.4061

If Control Group Is	Count	Mean	Different From Treatment Groups
1200	16	-3.304375	10, 0
60	16	-2.1575	
10	16	-0.429375	1200
0	18	-6.661338E-16	1200

Notes:
This section presents the results of two-sided comparisons of each group versus the control.
Only those groups that were different from the control group are listed.
Since the actual control group is not specified, a separate line is generated assuming
that each group is the control group. Only use the line of the report that uses the actual control group.

Duncan's Multiple-Comparison Test

Response: ECF_ALT
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=7.322288

Group	Count	Mean	Different From Groups
1200	16	-3.304375	10, 0
60	16	-2.1575	
10	16	-0.429375	1200
0	18	-6.661338E-16	1200

Notes:
This report provides multiple comparison tests for all pairwise differences between
the means. According to Hsu(1996, page 130), the specified family-wise error rate (alpha)
is overstated and the Tukey-Kramer method is recommended instead.

Tukey-Kramer Multiple-Comparison Test

Response: ECF_ALT
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=7.322288 Critical Value=3.7727

Group	Count	Mean	Different From Groups
1200	16	-3.304375	10, 0
60	16	-2.1575	
10	16	-0.429375	1200
0	18	-6.661338E-16	1200

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Tukey-Kramer's Simultaneous Confidence Intervals for Multiple Comparisons of All Pairs

Response: ECF_ALT
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=7.322288 Critical Value=3.7727

Comparison Groups	Count	Mean	Lower 95.0% Simult.C.I.	Mean Difference	Upper 95.0% Simult.C.I.	Test Result
0	**18**	**-6.661338E-16**				
- 10	16	-0.429375	-2.05093	0.429375	2.90968	
- 60	16	-2.1575	-0.3228046	2.1575	4.637805	
- 1200	16	-3.304375	0.8240704	3.304375	5.784679	U
10	**16**	**-0.429375**				
- 0	18	-6.661338E-16	-2.90968	-0.429375	2.05093	
- 60	16	-2.1575	-0.8240874	1.728125	4.280337	
- 1200	16	-3.304375	0.3227876	2.875	5.427212	U
60	**16**	**-2.1575**				
- 0	18	-6.661338E-16	-4.637805	-2.1575	0.3228046	
- 10	16	-0.429375	-4.280337	-1.728125	0.8240874	
- 1200	16	-3.304375	-1.405337	1.146875	3.699087	
1200	**16**	**-3.304375**				
- 0	18	-6.661338E-16	-5.784679	-3.304375	-0.8240704	L
- 10	16	-0.429375	-5.427212	-2.875	-0.3227876	L

Tukey-Kramer's Simultaneous Confidence Intervals for Multiple Comparisons of All Pairs

Response: ECF_ALT
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=7.322288 Critical Value=3.7727

Comparison Groups	Count	Mean	Lower 95.0% Simult.C.I.	Mean Difference	Upper 95.0% Simult.C.I.	Test Result
- 60	16	-2.1575	-3.699087	-1.146875	1.405337	

Notes:
This report provides joint simultaneous confidence intervals for all pairwise differences between the means.

Tukey-Kramer Multiple-Comparison Test

Response: BIS_ALT

Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=636.7239 Critical Value=3.7727

				Different From
	Group	Count	Mean	Groups
•	1200	16	856.9494	10, 0
	60	16	873.0944	
•	10	16	881.6569	1200
•	0	16	883.8075	1200

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: PATIENT	18	149.3316	8.2962	1.11	0.371095	
B: Time	3	26.76542	8.921808	1.19	0.321709	0.300438
S	48	358.389	7.466437			
Total (Adjusted)	69	534.7941				
Total	70					

* Term significant at alpha = 0.05

Means and Standard Error Section

Term	Count	Mean	Standard Error
All	70	-0.3982353	
A: PATIENT			
1	4	-1.8675	1.366239
2	4	1.4	1.366239
3	4	1.1	1.366239
4	4	0.6	1.366239
5	4	-1.38	1.366239
6	1	-0.3982353	2.732478
7	4	0.1425	1.366239
8	1	-0.3982353	2.732478
9	4	-3.2	1.366239
10	4	-0.855	1.366239
11	4	0.0775	1.366239
12	4	-0.0125	1.366239
13	4	0.0725	1.366239
14	4	1.4225	1.366239
15	4	-0.005	1.366239
16	4	-0.27	1.366239
17	4	-1.09	1.366239
18	4	1.0575	1.366239
19	4	-3.9625	1.366239
B: Time			
0	19	7.21645E-16	0.6268735
10	17	-0.7594118	0.6627233
60	17	-1.218235	0.6627233
1200	17	0.3847059	0.6627233

Analysis of Variance Report

Page/Date/Time 2 3/10/2013 11:04:55 AM
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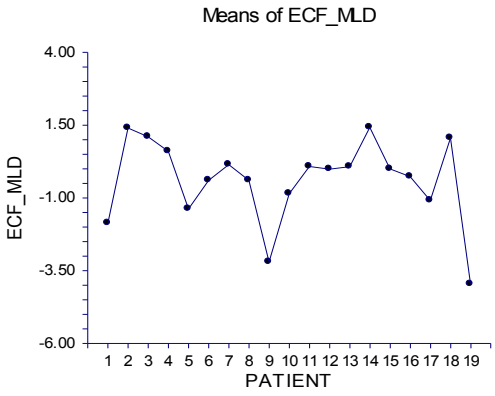
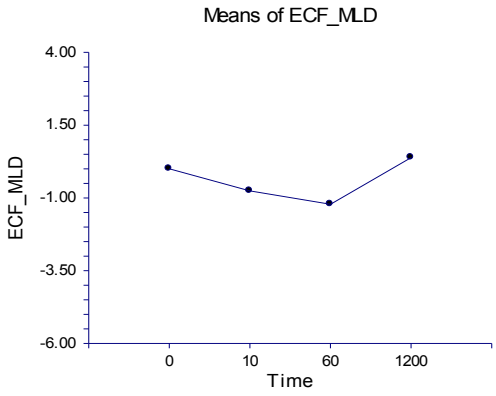
Response ECF_MLD

Plots Section





Bonferroni (All-Pairwise) Multiple Comparison Test

Response: ECF_MLD
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=48 MSE=7.466437 Critical Value=2.7520

Group	Count	Mean	Different From Groups
60	17	-1.218235	
10	17	-0.7594118	
0	19	7.21645E-16	
1200	17	0.3847059	

Notes:
This section presents the results of all paired comparisons among the means. Since this procedure uses the Bonferroni inequality, it is not as accurate as the Tukey-Kramer's method.

Dunnett's Two-Sided Multiple-Comparison Test With Control

Response: ECF_MLD
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=48 MSE=7.466437 Critical Value=2.4026

If Control Group Is	Count	Mean	Different From Treatment Groups
60	17	-1.218235	
10	17	-0.7594118	
0	19	7.21645E-16	
1200	17	0.3847059	

Notes:
This section presents the results of two-sided comparisons of each group versus the control.
Only those groups that were different from the control group are listed.
Since the actual control group is not specified, a separate line is generated assuming
that each group is the control group. Only use the line of the report that uses the actual control group.

Duncan's Multiple-Comparison Test

Response: ECF_MLD
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=48 MSE=7.466437

Group	Count	Mean	Different From Groups
60	17	-1.218235	
10	17	-0.7594118	
0	19	7.21645E-16	
1200	17	0.3847059	

Notes:
This report provides multiple comparison tests for all pairwise differences between
the means. According to Hsu(1996, page 130), the specified family-wise error rate (alpha)
is overstated and the Tukey-Kramer method is recommended instead.

Tukey-Kramer Multiple-Comparison Test

Response: ECF_MLD
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=48 MSE=7.466437 Critical Value=3.7638

Group	Count	Mean	Different From Groups
60	17	-1.218235	
10	17	-0.7594118	
0	19	7.21645E-16	
1200	17	0.3847059	

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Tukey-Kramer's Simultaneous Confidence Intervals for Multiple Comparisons of All Pairs

Response: ECF_MLD
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=48 MSE=7.466437 Critical Value=3.7638

Comparison Groups	Count	Mean	Lower 95.0% Simult.C.I.	Mean Difference	Upper 95.0% Simult.C.I.	Test Result
0	**19**	**7.21645E-16**				
- 10	17	-0.7594118	-1.668394	0.7594118	3.187217	
- 60	17	-1.218235	-1.20957	1.218235	3.646041	
- 1200	17	0.3847059	-2.812511	-0.3847059	2.0431	
10	**17**	**-0.7594118**				
- 0	19	7.21645E-16	-3.187217	-0.7594118	1.668394	
- 60	17	-1.218235	-2.03551	0.4588235	2.953156	
- 1200	17	0.3847059	-3.638451	-1.144118	1.350215	
60	**17**	**-1.218235**				
- 0	19	7.21645E-16	-3.646041	-1.218235	1.20957	
- 10	17	-0.7594118	-2.953156	-0.4588235	2.03551	
- 1200	17	0.3847059	-4.097274	-1.602941	0.8913918	
1200	**17**	**0.3847059**				
- 0	19	7.21645E-16	-2.0431	0.3847059	2.812511	
- 10	17	-0.7594118	-1.350215	1.144118	3.638451	

Tukey-Kramer's Simultaneous Confidence Intervals for Multiple Comparisons of All Pairs

Response: ECF_MLD
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=48 MSE=7.466437 Critical Value=3.7638

Comparison Groups	Count	Mean	Lower 95.0% Simult.C.I.	Mean Difference	Upper 95.0% Simult.C.I.	Test Result
- 60	17	-1.218235	-0.8913918	1.602941	4.097274	

Notes:
This report provides joint simultaneous confidence intervals for all pairwise differences between the means.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: PATIENT	18	197.9137	10.99521	1.63	0.088200	
B: Time	3	23.37518	7.791728	1.15	0.336800	0.291944
S	51	344.6609	6.758056			
Total (Adjusted)	72	566.3669				
Total	73					

* Term significant at alpha = 0.05

Means and Standard Error Section

Term	Count	Mean	Standard Error
All	73	-0.6502778	
A: PATIENT			
1	4	-0.4275	1.299813
2	4	-1.4325	1.299813
3	1	-0.6502778	2.599626
4	4	-3.0475	1.299813
5	4	-1.8025	1.299813
6	4	0.94	1.299813
7	4	-4.225	1.299813
8	4	1.415	1.299813
9	4	1.035	1.299813
10	4	-0.6875	1.299813
11	4	-1.37	1.299813
12	4	2.2375	1.299813
13	4	0.475	1.299813
14	4	-0.5925	1.299813
15	4	0.525	1.299813
16	4	-1.17	1.299813
17	4	-0.9375	1.299813
18	4	-3.1325	1.299813
19	4	0.4925	1.299813
B: Time			
0	19	-1.110223E-16	0.5963951
10	18	-1.387778	0.6127378
60	18	-1.011667	0.6127378
1200	18	-0.2016667	0.6127378

Analysis of Variance Report

Page/Date/Time 2 3/10/2013 11:06:06 AM
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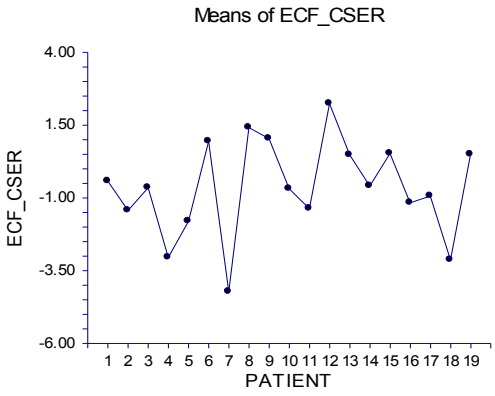
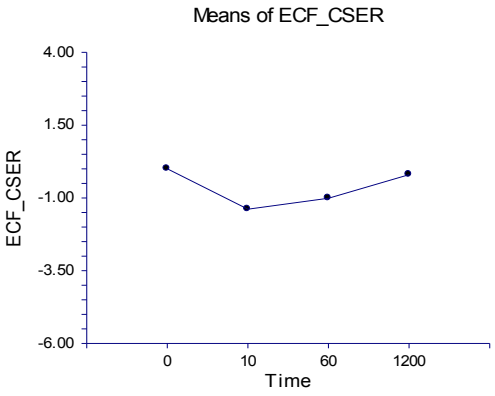
Response ECF_CSER

Plots Section





Bonferroni (All-Pairwise) Multiple Comparison Test

Response: ECF_CSER
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=51 MSE=6.758056 Critical Value=2.7451

Group	Count	Mean	Different From Groups
10	18	-1.387778	
60	18	-1.011667	
1200	18	-0.2016667	
0	19	-1.110223E-16	

Notes:
This section presents the results of all paired comparisons among the means. Since this procedure uses the Bonferroni inequality, it is not as accurate as the Tukey-Kramer's method.

Dunnett's Two-Sided Multiple-Comparison Test With Control

Response: ECF_CSER
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=51 MSE=6.758056 Critical Value=2.4011

If Control Group Is	Count	Mean	Different From Treatment Groups
10	18	-1.387778	
60	18	-1.011667	
1200	18	-0.2016667	
0	19	-1.110223E-16	

Notes:
This section presents the results of two-sided comparisons of each group versus the control.
Only those groups that were different from the control group are listed.
Since the actual control group is not specified, a separate line is generated assuming
that each group is the control group. Only use the line of the report that uses the actual control group.

Duncan's Multiple-Comparison Test

Response: ECF_CSER
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=51 MSE=6.758056

Group	Count	Mean	Different From Groups
10	18	-1.387778	
60	18	-1.011667	
1200	18	-0.2016667	
0	19	-1.110223E-16	

Notes:
This report provides multiple comparison tests for all pairwise differences between
the means. According to Hsu(1996, page 130), the specified family-wise error rate (alpha)
is overstated and the Tukey-Kramer method is recommended instead.

Tukey-Kramer Multiple-Comparison Test

Response: ECF_CSER
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=51 MSE=6.758056 Critical Value=3.7559

Group	Count	Mean	Different From Groups
10	18	-1.387778	
60	18	-1.011667	
1200	18	-0.2016667	
0	19	-1.110223E-16	

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Tukey-Kramer's Simultaneous Confidence Intervals for Multiple Comparisons of All Pairs

Response: ECF_CSER
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=51 MSE=6.758056 Critical Value=3.7559

Comparison Groups	Count	Mean	Lower 95.0% Simult.C.I.	Mean Difference	Upper 95.0% Simult.C.I.	Test Result
0	**19**	**-1.110223E-16**				
- 10	18	-1.387778	-0.8831159	1.387778	3.658671	
- 60	18	-1.011667	-1.259227	1.011667	3.28256	
- 1200	18	-0.2016667	-2.069227	0.2016667	2.47256	
10	**18**	**-1.387778**				
- 0	19	-1.110223E-16	-3.658671	-1.387778	0.8831159	
- 60	18	-1.011667	-2.677488	-0.3761111	1.925266	
- 1200	18	-0.2016667	-3.487488	-1.186111	1.115266	
60	**18**	**-1.011667**				
- 0	19	-1.110223E-16	-3.28256	-1.011667	1.259227	
- 10	18	-1.387778	-1.925266	0.3761111	2.677488	
- 1200	18	-0.2016667	-3.111377	-0.81	1.491377	
1200	**18**	**-0.2016667**				
- 0	19	-1.110223E-16	-2.47256	-0.2016667	2.069227	
- 10	18	-1.387778	-1.115266	1.186111	3.487488	

Tukey-Kramer's Simultaneous Confidence Intervals for Multiple Comparisons of All Pairs

Response: ECF_CSER
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=51 MSE=6.758056 Critical Value=3.7559

Comparison Groups	Count	Mean	Lower 95.0% Simult.C.I.	Mean Difference	Upper 95.0% Simult.C.I.	Test Result
- 60	18	-1.011667	-1.491377	0.81	3.111377	

Notes:
This report provides joint simultaneous confidence intervals for all pairwise differences between the means.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: PATIENT	18	169.0128	9.389601	2.52	0.006147*	
B: Time	3	19.14537	6.381789	1.71	0.177638	0.417665
S	45	167.567	3.72371			
Total (Adjusted)	66	355.9085				
Total	67					

* Term significant at alpha = 0.05

Means and Standard Error Section

Term	Count	Mean	Standard Error
All	67	-0.2529688	
A: PATIENT			
1	4	-1.825	0.9648458
2	4	1.8925	0.9648458
3	1	-0.2529688	1.929692
4	4	-0.1175	0.9648458
5	4	-1.92	0.9648458
6	1	-0.2529688	1.929692
7	4	-1.7725	0.9648458
8	1	-0.2529688	1.929692
9	4	-0.9	0.9648458
10	4	1.04	0.9648458
11	4	1.345	0.9648458
12	4	-1.46	0.9648458
13	4	0.035	0.9648458
14	4	-1.5325	0.9648458
15	4	1.3275	0.9648458
16	4	-0.65	0.9648458
17	4	1.9875	0.9648458
18	4	1.905	0.9648458
19	4	-3.4025	0.9648458
B: Time			
0	19	7.771561E-16	0.4427016
10	16	-0.035625	0.4824229
60	16	-1.18625	0.4824229
1200	16	0.21	0.4824229

Analysis of Variance Report

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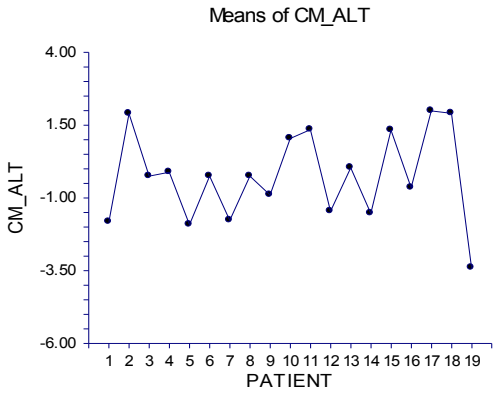
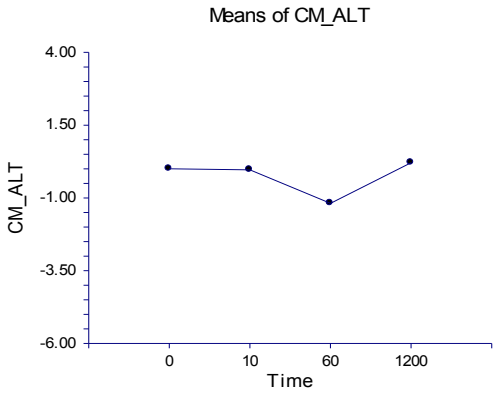
Response CM_ALT

Plots Section





Bonferroni (All-Pairwise) Multiple Comparison Test

Response: CM_ALT
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=3.72371 Critical Value=2.7599

Group	Count	Mean	Different From Groups
60	16	-1.18625	
10	16	-0.035625	
0	19	7.771561E-16	
1200	16	0.21	

Notes:
This section presents the results of all paired comparisons among the means. Since this procedure uses the Bonferroni inequality, it is not as accurate as the Tukey-Kramer's method.

Dunnett's Two-Sided Multiple-Comparison Test With Control

Response: CM_ALT
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=3.72371 Critical Value=2.4043

If Control Group Is	Count	Mean	Different From Treatment Groups
60	16	-1.18625	
10	16	-0.035625	
0	19	7.771561E-16	
1200	16	0.21	

Notes:
This section presents the results of two-sided comparisons of each group versus the control.
Only those groups that were different from the control group are listed.
Since the actual control group is not specified, a separate line is generated assuming
that each group is the control group. Only use the line of the report that uses the actual control group.

Duncan's Multiple-Comparison Test

Response: CM_ALT
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=3.72371

Group	Count	Mean	Different From Groups
60	16	-1.18625	
10	16	-0.035625	
0	19	7.771561E-16	
1200	16	0.21	

Notes:
This report provides multiple comparison tests for all pairwise differences between
the means. According to Hsu(1996, page 130), the specified family-wise error rate (alpha)
is overstated and the Tukey-Kramer method is recommended instead.

Tukey-Kramer Multiple-Comparison Test

Response: CM_ALT
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=3.72371 Critical Value=3.7727

Group	Count	Mean	Different From Groups
60	16	-1.18625	
10	16	-0.035625	
0	19	7.771561E-16	
1200	16	0.21	

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Tukey-Kramer's Simultaneous Confidence Intervals for Multiple Comparisons of All Pairs

Response: CM_ALT
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=3.72371 Critical Value=3.7727

Comparison Groups	Count	Mean	Lower 95.0% Simult.C.I.	Mean Difference	Upper 95.0% Simult.C.I.	Test Result
0	**19**	**7.771561E-16**				
- 10	16	-0.035625	-1.711096	0.035625	1.782346	
- 60	16	-1.18625	-0.5604711	1.18625	2.932971	
- 1200	16	0.21	-1.956721	-0.21	1.536721	
10	**16**	**-0.035625**				
- 0	19	7.771561E-16	-1.782346	-0.035625	1.711096	
- 60	16	-1.18625	-0.6694168	1.150625	2.970667	
- 1200	16	0.21	-2.065667	-0.245625	1.574417	
60	**16**	**-1.18625**				
- 0	19	7.771561E-16	-2.932971	-1.18625	0.5604711	
- 10	16	-0.035625	-2.970667	-1.150625	0.6694168	
- 1200	16	0.21	-3.216292	-1.39625	0.4237918	
1200	**16**	**0.21**				
- 0	19	7.771561E-16	-1.536721	0.21	1.956721	
- 10	16	-0.035625	-1.574417	0.245625	2.065667	

Tukey-Kramer's Simultaneous Confidence Intervals for Multiple Comparisons of All Pairs

Response: CM_ALT
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=45 MSE=3.72371 Critical Value=3.7727

Comparison Groups	Count	Mean	Lower 95.0% Simult.C.I.	Mean Difference	Upper 95.0% Simult.C.I.	Test Result
- 60	16	-1.18625	-0.4237918	1.39625	3.216292	

Notes:
This report provides joint simultaneous confidence intervals for all pairwise differences between the means.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: PATIENT	18	93.11005	5.172781	2.07	0.023146*	
B: Time	3	11.09998	3.699994	1.48	0.231704	0.366594
S	48	119.9701	2.499378			
Total (Adjusted)	69	224.2076				
Total	70					

* Term significant at alpha = 0.05

Means and Standard Error Section

Term	Count	Mean	Standard Error
All	70	0.1186765	
A: PATIENT			
1	4	0.415	0.7904711
2	4	-1.23	0.7904711
3	4	-0.0275	0.7904711
4	4	-0.3725	0.7904711
5	4	-0.8975	0.7904711
6	1	0.1186765	1.580942
7	4	-1.6025	0.7904711
8	1	0.1186765	1.580942
9	4	0.335	0.7904711
10	4	-0.6725	0.7904711
11	4	-0.05	0.7904711
12	4	0.02	0.7904711
13	4	3.59	0.7904711
14	4	-0.1975	0.7904711
15	4	2.15	0.7904711
16	4	-0.0025	0.7904711
17	4	0.13	0.7904711
18	4	0.43	0.7904711
19	4	7.21645E-16	0.7904711
B: Time			
0	19	-6.106227E-16	0.362693
10	17	-0.3252941	0.3834348
60	17	2.352941E-02	0.3834348
1200	17	0.7764706	0.3834348

Analysis of Variance Report

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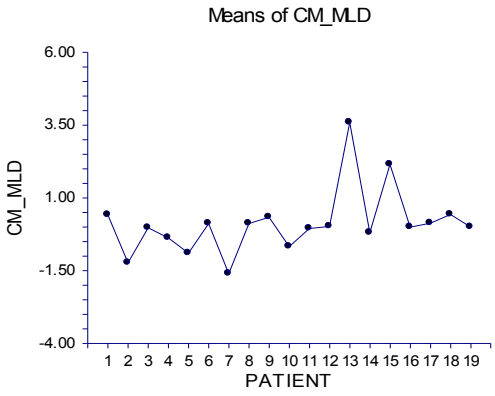
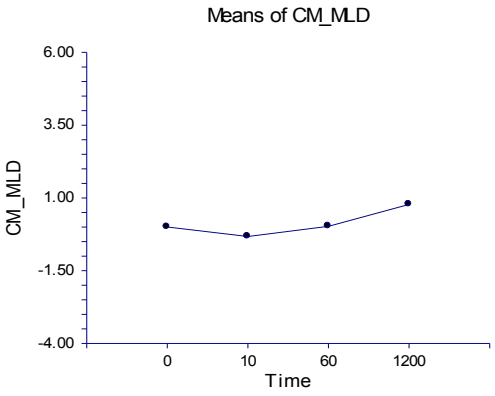
Response CM_MLD

Plots Section





Bonferroni (All-Pairwise) Multiple Comparison Test

Response: CM_MLD
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=48 MSE=2.499378 Critical Value=2.7520

Group	Count	Mean	Different From Groups
10	17	-0.3252941	
0	19	-6.106227E-16	
60	17	2.352941E-02	
1200	17	0.7764706	

Notes:
This section presents the results of all paired comparisons among the means. Since this procedure uses the Bonferroni inequality, it is not as accurate as the Tukey-Kramer's method.

Dunnett's Two-Sided Multiple-Comparison Test With Control

Response: CM_MLD
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=48 MSE=2.499378 Critical Value=2.4026

If Control Group Is	Count	Mean	Different From Treatment Groups
10	17	-0.3252941	
0	19	-6.106227E-16	
60	17	2.352941E-02	
1200	17	0.7764706	

Notes:
This section presents the results of two-sided comparisons of each group versus the control.
Only those groups that were different from the control group are listed.
Since the actual control group is not specified, a separate line is generated assuming
that each group is the control group. Only use the line of the report that uses the actual control group.

Duncan's Multiple-Comparison Test

Response: CM_MLD
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=48 MSE=2.499378

Group	Count	Mean	Different From Groups
10	17	-0.3252941	
0	19	-6.106227E-16	
60	17	2.352941E-02	
1200	17	0.7764706	

Notes:
This report provides multiple comparison tests for all pairwise differences between
the means. According to Hsu(1996, page 130), the specified family-wise error rate (alpha)
is overstated and the Tukey-Kramer method is recommended instead.

Tukey-Kramer Multiple-Comparison Test

Response: CM_MLD
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=48 MSE=2.499378 Critical Value=3.7638

Group	Count	Mean	Different From Groups
10	17	-0.3252941	
0	19	-6.106227E-16	
60	17	2.352941E-02	
1200	17	0.7764706	

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Tukey-Kramer's Simultaneous Confidence Intervals for Multiple Comparisons of All Pairs

Response: CM_MLD
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=48 MSE=2.499378 Critical Value=3.7638

Comparison Groups	Count	Mean	Lower 95.0% Simult.C.I.	Mean Difference	Upper 95.0% Simult.C.I.	Test Result
0	**19**	**-6.106227E-16**				
- 10	17	-0.3252941	-1.079372	0.3252941	1.72996	
- 60	17	2.352941E-02	-1.428196	-2.352941E-02	1.381137	
- 1200	17	0.7764706	-2.181137	-0.7764706	0.6281957	
10	**17**	**-0.3252941**				
- 0	19	-6.106227E-16	-1.72996	-0.3252941	1.079372	
- 60	17	2.352941E-02	-1.791981	-0.3488235	1.094334	
- 1200	17	0.7764706	-2.544922	-1.101765	0.3413927	
60	**17**	**2.352941E-02**				
- 0	19	-6.106227E-16	-1.381137	2.352941E-02	1.428196	
- 10	17	-0.3252941	-1.094334	0.3488235	1.791981	
- 1200	17	0.7764706	-2.196099	-0.7529412	0.6902162	
1200	**17**	**0.7764706**				
- 0	19	-6.106227E-16	-0.6281957	0.7764706	2.181137	
- 10	17	-0.3252941	-0.3413927	1.101765	2.544922	

Tukey-Kramer's Simultaneous Confidence Intervals for Multiple Comparisons of All Pairs

Response: CM_MLD
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=48 MSE=2.499378 Critical Value=3.7638

Comparison Groups	Count	Mean	Lower 95.0% Simult.C.I.	Mean Difference	Upper 95.0% Simult.C.I.	Test Result
- 60	17	2.352941E-02	-0.6902162	0.7529412	2.196099	

Notes:
This report provides joint simultaneous confidence intervals for all pairwise differences between the means.

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: PATIENT	18	185.1591	10.28662	3.31	0.000407*	
B: Time	3	3.3159	1.1053	0.36	0.785494	0.114832
S	51	158.6568	3.110918			
Total (Adjusted)	72	347.2608				
Total	73					

* Term significant at alpha = 0.05

Means and Standard Error Section

Term	Count	Mean	Standard Error
All	73	0.3616667	
A: PATIENT			
1	4	0.6175	0.8818897
2	4	-0.28	0.8818897
3	1	0.3616667	1.763779
4	4	1.2475	0.8818897
5	4	-3.7775	0.8818897
6	4	-0.1625	0.8818897
7	4	3.31	0.8818897
8	4	1.0525	0.8818897
9	4	2.1	0.8818897
10	4	0.8875	0.8818897
11	4	-1.5675	0.8818897
12	4	0.4	0.8818897
13	4	1.7675	0.8818897
14	4	-0.9575	0.8818897
15	4	2.4675	0.8818897
16	4	0.77	0.8818897
17	4	-1.1525	0.8818897
18	4	0.33	0.8818897
19	4	-0.5425	0.8818897
B: Time			
0	19	0	0.4046387
10	18	0.4066667	0.4157268
60	18	0.495	0.4157268
1200	18	0.545	0.4157268

Analysis of Variance Report

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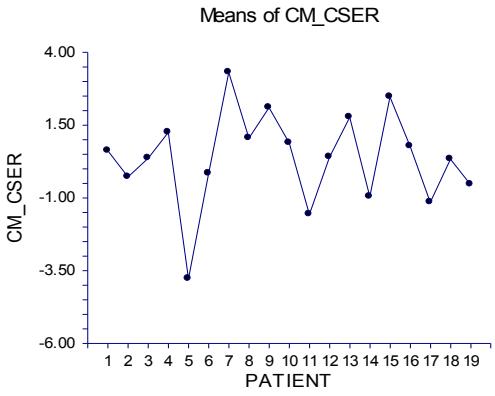
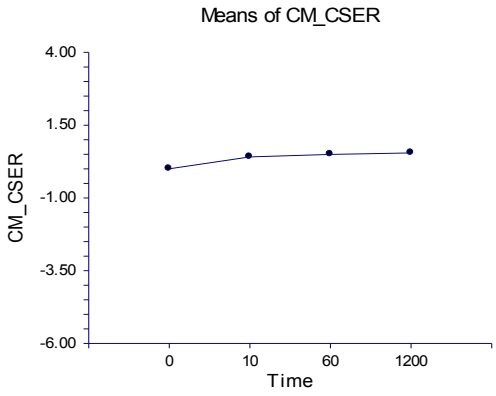
Response CM_CSER

Plots Section





Bonferroni (All-Pairwise) Multiple Comparison Test

Response: CM_CSER
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=51 MSE=3.110918 Critical Value=2.7451

Group	Count	Mean	Different From Groups
0	19	0	
10	18	0.4066667	
60	18	0.495	
1200	18	0.545	

Notes:
This section presents the results of all paired comparisons among the means. Since this procedure uses the Bonferroni inequality, it is not as accurate as the Tukey-Kramer's method.

Dunnett's Two-Sided Multiple-Comparison Test With Control

Response: CM_CSER
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=51 MSE=3.110918 Critical Value=2.4011

If Control Group Is	Count	Mean	Different From Treatment Groups
0	19	0	
10	18	0.4066667	
60	18	0.495	
1200	18	0.545	

Notes:
This section presents the results of two-sided comparisons of each group versus the control.
Only those groups that were different from the control group are listed.
Since the actual control group is not specified, a separate line is generated assuming
that each group is the control group. Only use the line of the report that uses the actual control group.

Duncan's Multiple-Comparison Test

Response: CM_CSER
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=51 MSE=3.110918

Group	Count	Mean	Different From Groups
0	19	0	
10	18	0.4066667	
60	18	0.495	
1200	18	0.545	

Notes:
This report provides multiple comparison tests for all pairwise differences between
the means. According to Hsu(1996, page 130), the specified family-wise error rate (alpha)
is overstated and the Tukey-Kramer method is recommended instead.

Tukey-Kramer Multiple-Comparison Test

Response: CM_CSER
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=51 MSE=3.110918 Critical Value=3.7559

Group	Count	Mean	Different From Groups
0	19	0	
10	18	0.4066667	
60	18	0.495	
1200	18	0.545	

Notes:
This report provides multiple comparison tests for all pairwise differences between the means.

Tukey-Kramer's Simultaneous Confidence Intervals for Multiple Comparisons of All Pairs

Response: CM_CSER
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=51 MSE=3.110918 Critical Value=3.7559

Comparison Groups	Count	Mean	Lower 95.0% Simult.C.I.	Mean Difference	Upper 95.0% Simult.C.I.	Test Result
0	**19**	**0**				
- 10	18	0.4066667	-1.94741	-0.4066667	1.134076	
- 60	18	0.495	-2.035743	-0.495	1.045743	
- 1200	18	0.545	-2.085743	-0.545	0.9957429	
10	**18**	**0.4066667**				
- 0	19	0	-1.134076	0.4066667	1.94741	
- 60	18	0.495	-1.649758	-8.833333E-02	1.473092	
- 1200	18	0.545	-1.699758	-0.1383333	1.423092	
60	**18**	**0.495**				
- 0	19	0	-1.045743	0.495	2.035743	
- 10	18	0.4066667	-1.473092	8.833333E-02	1.649758	
- 1200	18	0.545	-1.611425	-0.05	1.511425	
1200	**18**	**0.545**				
- 0	19	0	-0.9957429	0.545	2.085743	
- 10	18	0.4066667	-1.423092	0.1383333	1.699758	

Tukey-Kramer's Simultaneous Confidence Intervals for Multiple Comparisons of All Pairs

Response: CM_CSER
Term B: Time

Alpha=0.050 Error Term=S(AB) DF=51 MSE=3.110918 Critical Value=3.7559

Comparison Groups	Count	Mean	Lower 95.0% Simult.C.I.	Mean Difference	Upper 95.0% Simult.C.I.	Test Result
- 60	18	0.495	-1.511425	0.05	1.611425	

Notes:
This report provides joint simultaneous confidence intervals for all pairwise differences between the means.