Imaging Biomarkers of Lymphatic Dysfunction

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1.0 Background

Breast cancer treatment-related lymphedema (BCRL) is a chronic, debilitating disease caused by lymphatic flow obstruction and lymphedema of the upper limb and torso secondary to necessary cancer therapies, namely lymph node removal. BCRL has been reported to occur in 20-30% of breast cancer survivors following these therapies, which is in addition to similar incidence of secondary lymphedema in the upper limb occurring in persons with other therapies necessitating axillary lymph node removal and/or radiation. However, owing to a lack of methodology for sensitively identifying lymphatic system compromise, there are important gaps in our knowledge regarding which patients are at highest risk and how and when therapies should be applied to minimize impairment. Here, novel and noninvasive magnetic resonance imaging approaches for measuring lymphatic dysfunction are applied to improve procedures for preventing, predicting, and treating secondary lymphedema.

The long-term goal of this work is to develop and apply novel tools to visualize lymphatic dysfunction, and also to incorporate these tools to provide new observables that can be used as much needed trial endpoints to optimize therapies and improve patient outcomes. This research initiative which started at VUMC over four years ago has led to the development of new, noninvasive imaging methods for evaluating LN function and morphology here at VUMC, along with approaches for visualizing lymphatic vessels and tissue impairment without exogenous contrast agents. These methods have been applied by us in healthy adults across the lifespan to evaluate normative parameter ranges, in surveillance imaging of BCRL patients at multiple disease stages, and in BCRL patients before and after manual lymphatic drainage (MLD) therapy[1-4]. This work has led to the development of multiple new biomarkers that are more sensitive than traditional external measures of lymphatic dysfunction.

We will build on the development efforts from our previous and ongoing lymphedema research (IRB #131172) to apply methods to evaluate lymphatic dysfunction in multiple stages of secondary lymphedema management, including (i) prevention, (ii) sub-clinical risk identification, and (iii) therapy.

Below summarizes this study's overall significance:

1. We will utilize multi-parametric anatomical and molecular imaging of LNs, which incorporate measures of anatomy and chemical environment, to evaluate abilities to discriminate metastatic from benign LNs noninvasively in vivo. These approaches have already been applied in other applications of malignancy[5-7], and we have developed innovative high spatial resolution variants to enable sub-millimeter LN characterization in vivo[1]. If successful, findings would have relevance for noninvasively mapping the topography of LN malignancy, which could initiate future investigations to ultimately inform surgery decisions and reduce unnecessary LN removal.

2. We will extend our current trial to evaluate sub-clinical internal and external risk factors of secondary lymphedema progression in the arm and upper torso following cancer therapies post-LN removal and test more refined hypotheses regarding how impaired lymphatic pumping, superficial axillary water accumulation, and interstitial adipose accumulation portend secondary lymphedema onset and progression. This work will be significant for understanding prognostic potential of the new internal markers relative to existing risk factors, and it will reinforce using these measures to triage patients for disease-modifying therapies or as endpoints in therapeutic trials.

3. Emerging therapies that utilize CDT with refined approaches in the individual components (i.e. incorporating graded negative pressure) are being utilized in specialized lymphedema outpatient clinics including Vanderbilt Lymphedema Clinic, but formal efficacy trials have not been performed. We will perform a multiple-measures cross-over trial to evaluate the impact of CDT with vs. without graded negative pressure on reducing lymph stasis and internal markers of fibrosis in chronic BCRL states, along with a subgroup of 1 time treatment before and after MRI and nonMRI measures for immediate treatment impact investigation. Improved lymphatic flow has demonstrated the ability to induce lymphangiogenesis in pre-clinical studies[8], and it will be crucial to demonstrate internal improvements in vivo, relative to basic symptomatology changes which can be subjective and difficult to quantify, to underscore the need for expanding healthcare coverage of these disease-modifying therapies.

4. The imaging methods developed evaluate functional and anatomical lymphatic system properties and will be applied in hypothesis-driven studies of BCRL in this work. Owing to the known etiology of overt secondary lymphedema such as BCRL, this condition represents an ideal system to evaluate relationships between tissue physiology, such as adiposity and interstitial protein accumulation, and lymphatic insufficiency. However, the findings should have relevance for improving our understanding of the physiological changes that occur in lymphatic vessels and dependent tissues in other cases of lymphedema or disorders where lymphatic dysfunction is suspected[9].

2.0 Rationale and Specific Aims

The goal of this work is to apply novel, noninvasive magnetic resonance imaging (MRI) methods for visualizing lymphatic circulation dysfunction to test fundamental hypotheses about secondary lymphedema risk factors and therapies. Breast cancer treatment-related lymphedema (BCRL) arises secondary to surgical axillary lymph node (LN) dissection and irritation, and is a chronic and lifelong condition affecting a high 21.4% of patients receiving common breast cancer therapies. Persons at risk for secondary lymphedema following axillary lymph node removal also include undergoing treatment for other cancers, such as melanoma involving the upper body, necessitating the need for axially

lymph node removal. Reducing condition onset and improving management represent major unmet clinical needs, and emerging efforts focus on improving quality of life through more informed LN dissection and biopsy decisions, optimizing post-surgical complex decongestive therapy (CDT), and exploring novel pharmacological and surgical procedures. However, fundamental gaps in our knowledge persist regarding optimized implementation of these therapies and details of the physiological changes they elicit. The major underlying limitation is that there is a shortage of imaging methods available that can be used to evaluate lymphatic function directly, and there is currently no consensus regarding effective outcome measures for therapeutic efficacy evaluation. Rather, LN removal is frequently based on sentinel LN biopsy and additional subjectivity of the surgeon. Therapy evaluation is frequently based on coarse measurements such as changes in limb volume or patient-reported symptoms, which provide little information on underlying mechanistic changes that could be used to further refine these therapies. As part of our ongoing INFORM clinical trial of lymphedema progression and therapy, we have demonstrated potential for new, noninvasive MRI approaches to identify lymphedema risk in sub-clinical disease stages, as well as to visualize internal changes in lymphatic functioning as a result of emerging therapies. Here, we propose to extend these studies to improve abilities for lymphedema theranostics.

Aim 1: Prevention. We will apply new anatomical and functional LN imaging approaches to identify LN profiles specific to biopsy-confirmed metastatic LNs; findings could be used to better inform LN removal and reduce the incidence of benign LN dissection. We will incorporate healthy female controls to serve as a comparison to the patient population.

Aim 2: Progression. We will improve our understanding of secondary lymphedema risk progression following cancer therapy by testing the hypothesis that superficial tissue profiles and subcutaneous adipose accumulation are more prevalent in patients experiencing lymphedema progression, and thereby could be used to triage patients for aggressive therapies prior to overt symptoms and irreversible damage.

Aim 3: Therapy. We will perform a repeated-measures cross-over trial to test the hypothesis that mobilization of protein enriched hardened tissue using graded negative pressure therapy in conjunction with complete decongestive therapy (CDT) is more effective to standard CDT alone for secondary lymphedema management. Both of these conservative physical therapy treatments are commonly employed for treatment of secondary lymphedema and the Vanderbilt Lymphedema Clinic utilizes the graded negative pressure therapy as a component of treatment. Additionally we will perform a subgroup of 1-time only hour treatment with before and after MRI and nonMRI measurements for additional immediate treatment impact in CDT, CDT with graded negative pressure, or exercise and self management treatment strategies alone as commonly performed in outpatient physical therapy clinics and at home for the management of BCRL.

The overarching goal is to develop objective markers of lymphatic dysfunction that can be used in emerging therapeutic trials of cancer and lymphedema therapies to be used to reduce both the incidence and severity of symptoms associated with this prevalent and chronic condition.

Aim (1). To compare *in vivo* anatomical and novel molecular imaging contrasts between LNs with versus without biopsy-confirmed metastasis.

Hypothesis (1). Biochemical LN profiles quantified from noninvasive chemical exchange saturation transfer (CEST) MRI provide discriminatory potential for distinguishing metastatic versus benign LNs pre-operatively. These biochemical profiles are more discriminatory than anatomical measures of LN morphology.

Aim (2). To identify sub-clinical biomarkers of lymphatic insufficiency using quantitative evaluation of lymphatic vessel anatomy, axillary edema, and interstitial adiposity.

Hypothesis (2). Sub-acute internal imaging markers reflective of tissue microenvironment and interstitial adipose accumulation provide superior discriminatory abilities for portending BCRL progression compared with established risk factors such as BMI, age, and quantity of LNs removed.

Aim (3). To evaluate the impact of CDT with and without graded negative pressure therapy on internal measures of lymphatic dysfunction and edema in patients with chronic BCRL.

Hypothesis (3). Mobilization of protein-enriched hardened tissue using graded negative pressure therapy in conjunction with CDT will reduce (i) superficial markers of interstitial protein accumulation and (ii) lymph stasis, and improve (iii) patient-valued functional outcomes compared to standard CDT and/or exercise and self management treatment strategies.

3.0 Inclusion/Exclusion Criteria

The following subjects will be excluded from all aims of this study:

1. Subjects who have any type of non-MRI compatible bioimplant activated by mechanical, electronic, or magnetic means (e.g., cochlear implants, pacemakers, neurostimulators, biostimulators, electronic infusion pumps, etc.), because such devices may be displaced or malfunction.
2. Subjects who have any type of ferromagnetic bioimplant that could potentially be displaced.
3. Subjects who have cerebral aneurysm clips.

4. Subjects who may have shrapnel imbedded in their bodies (such as from war wounds), metal workers and machinists (potential for metallic fragments in or near the eyes).
5. Minors (younger than 18 years)
6. Pregnant women
7. Subjects who have open wounds on either wrist and / or right ankle because of contraindications with placement of electrodes to obtain the L-DEX U400 readings.

Also excluded are subjects incapable of giving informed written consent:

1. Subjects who are non-English speaking, Children/minors* (Form #1117), Pregnant women/fetal tissue/placenta* (Form #1116), Cognitively impaired* (Form #1118), Prisoners* (Form #1115)
2. Subjects who cannot adhere to the experimental protocols for any reason, or have an inability to communicate with the researcher.
3. Subjects who have limited mental ability to give informed consent, mentally retarded, altered mental status, mental disability, confusion, or psychiatric disorders.
4. Prisoners

To minimize discomfort, we will also exclude subjects who exhibit noticeable anxiety and/or claustrophobia or who exhibit severe vertigo when they are moved into the magnet.

Inclusion criteria for Aim 1:

1. Females (over the age of 18 years) planning to have unilateral or bilateral axillary lymph node removal surgery as a component of their cancer regime
2. or Females (over the age of 18 years) without history of cancer or lymphedema to serve as study controls

Exclusion criteria for Aims 1:

1. Any contraindication to MRI.
2. Any axillary lymph node removal procedure already performed before study visit.
3. Persons who are deemed clinically unsuitable for an MRI by their treating physician
4. Severe claustrophobia
5. Minors (younger than 18 years)
6. To minimize discomfort, we will also exclude subjects who exhibit noticeable anxiety and/or claustrophobia or who exhibit severe vertigo when they are moved into the magnet. All participants will be screened with the safety screening form currently used for research studies on all research MRI scanner
7. Open wounds on right ankle or bilateral wrists for purposes of obtaining external L-Dex extracellular fluid reading.

8. Subjects incapable of giving informed written consent:
    a. Subjects who are non-English speaking
    b. Subjects who cannot adhere to the experimental protocols for any reason, or have an inability to communicate with the researcher.
    c. Subjects who have limited mental ability to give informed consent, mentally retarded, altered mental status, mental disability, confusion, or psychiatric disorders.
9. Prisoners

Inclusion criteria for Aims 2:

1. Females (over the age of 18 years) who have already had unilateral axillary lymph node removal surgery as a component of their cancer regime in the "at risk" side being monitored for lymphedema development.

Exclusion criteria for Aims 2:

1. Any contraindication to MRI.
2. Persons with signs or symptoms of lymphedema before their first MRI study visit for their "at risk side" being monitored for lymphedema development.
3. Persons who are deemed clinically unsuitable for an MRI by their treating physician
4. Severe claustrophobia
5. Minors (younger than 18 years)
6. To minimize discomfort, we will also exclude subjects who exhibit noticeable anxiety and/or claustrophobia or who exhibit severe vertigo when they are moved into the magnet. All participants will be screened with the safety screening form currently used for research studies on all research MRI scanners.
7. Open wounds on right ankle or bilateral wrists for purposes of obtaining external L-Dex extracellular fluid reading.
8. Also excluded are subjects incapable of giving informed written consent:
    a. Subjects who are non-English speaking
    b. Subjects who cannot adhere to the experimental protocols for any reason, or have an inability to communicate with the researcher.
    c. Subjects who have limited mental ability to give informed consent, mentally retarded, altered mental status, mental disability, confusion, or psychiatric disorders.
9. Prisoners

Inclusion criteria for Aim 3:

1. Females (over the age of 18 years) diagnosed with unilateral secondary lymphedema in the upper quadrant and/or extremity following cancer related surgery involving removal of any axillary lymph nodes and/or radiation therapy.

Exclusion criteria for Aims 3:

1. Any contraindication to MRI.
2. Persons who have undergone bilateral axillary lymph node removal and thus are at risk for bilateral secondary lymphedema
3. Persons who are deemed clinically unsuitable for an MRI or lymphedema therapy by their treating physician
4. Severe claustrophobia
5. Minors (younger than 18 years)
6. To minimize discomfort, we will also exclude subjects who exhibit noticeable anxiety and/or claustrophobia or who exhibit severe vertigo when they are moved into the magnet. All participants will be screened with the safety screening form currently used for research studies on all research MRI scanners and the manual lymphatic drainage screening form administered by a licensed lymphedema physical therapist.
7. Open wounds on right ankle or bilateral wrists for purposes of obtaining external L-Dex extracellular fluid reading.
8. Also excluded are subjects incapable of giving informed written consent:
9. Subjects who are non-English speaking
    a. Subjects who cannot adhere to the experimental protocols for any reason, or have an inability to communicate with the researcher.
    b. Subjects who have limited mental ability to give informed consent, mentally retarded, altered mental status, mental disability, confusion, or psychiatric disorders.
10. Prisoners

4.0 Enrollment / Randomization

1. Subjects with a clinical diagnosis of lymphedema following axillary lymph node removal including breast cancer related surgery will be made aware of the study by recruitment flyers and / or their health care clinician, who will ask the patient if they would like for a member of the research team to contact them regarding potential participation in the research study. If the patient would like to receive more information about participating in the study and like to be contacted by a KSP in the study, then the clinician will provide the patient's preferred contact information to a KSP member. The patient will then be contacted by the KSP member and the risks and benefits of the study will be described. Importantly, the treating clinician will only ask if the patient would like to receive more information about the study, but will not be involved in obtaining consent or coercing the patient to participate and the patient will understand there is no change in their clinician care regardless of participation or not in the study. Furthermore, to recruit patients receiving these cancer therapies in local

nonVUMC clinics, we will contact the local clinics using the IRB approved letter and distribute IRB approved flyers to the clinics. Additional patients will be identified using the lymphedema research database where eligible persons request to be on the database to be informed when potential studies become available for the KSP to contact them regarding participation.

2. For Aim 1 and 2 of the study tracking patients near the time of their breast cancer related surgery, we will recruit patients from the One Hundred Oaks Breast Center outpatient clinics seen by the VUMC surgical oncologists and oncologists where the patients are seen for their cancer care. Furthermore, to recruit patients receiving these cancer surgeries in local non-VUMC cancer centers, we will contact the local clinics using the IRB approved letter and distribute IRB approved flyers to the clinics.
3. We will also utilize research databases additional recruitment tools for this protocol. ResearchMatch.org will be utilized as a recruitment tool for this protocol. ResearchMatch.org is a national electronic, web-based recruitment tool that was created through the Clinical & Translational Science Awards Consortium in 2009 and is maintained at Vanderbilt University as an IRB-approved data repository **(*see IRB #090207*)**. SUBJECT LOCATOR will be used as a recruitment tool to identify potential research subjects based on data available in VU clinical systems (e.g. STAR Panel, WizOrder, Clinic Scheduling). The SUBJECT LOCATOR program is part of a toolset available through VICTR that enables teams to specify inclusion/exclusion criteria for a specific study. The inclusion/exclusion criteria are codified for computable use and combined with data coming through VU Clinical Systems to proactively identify individuals who might qualify for a study. Once a 'match' is made, research study personnel are alerted using confidential messaging or a secure web portal and they may then use the information to further review the patient's information using the Vanderbilt Electronic Medical Record. If the patient is further deemed a candidate for the study, the study personnel will notify the patient's care provider who will then ask the patient if they would be interested in communicating with study personnel about research population.
4. IRB approved flyers/advertisements of the study may also be distributed to outpatient clinics and lymphedema support groups/networks as additional means of subject recruitment for the aims of the study.
5. Recruitment of patients will be done through flyers, posted adverts, research match, email, departmental research boards, and those who contact KSP directly using our VUMC website link for interested patient volunteers (https://ww2.mc.vanderbilt.edu/donahuelab/50860).
6. Reporting Workbench Reports (RWB) are available/viewable in eStar. These reports are developed using real-time data and can be customized to meet study-specific inclusion/exclusion criteria. Research team members with eStar access and access to the Report Groups can view/run the reports

as frequently as needed. While the report is generally limited to certain variables, it provides easy access to a patient's record for additional screening and confirmation of eligibility.

A custom RWB report will be utilized to identify potential participants. The report will filter on study inclusion/exclusion criteria computable in the EHR and EMR . Additionally, the report will include OK to Contact status, MHAV account status, and ability to receive MHAV messages. The report may also display additional information that will facilitate screening. We will utilize these systems for MHAV mass messaging and Individual Direct to Patient Messaging for out-reach communication.

5.0 Study procedures

1. Informed consent will be obtained via e-consent initially and then during initial visit reviewed in a private setting (e.g., an otherwise empty conference room or waiting area) by one of the study Investigators. This initial e-consent will occur as far in advance of the exam as possible, preferably before the day of the study and a KSP will review any questions with patient remotely as needed prior to onset of study. The study participant will be given a copy of the signed consent form at first visit. The subject will be screened for the study inclusion and exclusion criteria including MRI contraindications or MLD contraindications or precautions to determine eligibility of the study.

2. All subjects will complete an MRI screening form in advance of their scheduled visits for any MRI. The MRI screening form will be cleared by the KSPs and MRI technicians prior to scheduling their 1st MRI visit. This MRI screening form will be completed for each MRI scan a subject may have during the course of the study.

3. The informed consent andthe MRI screening form will be sent electronically via email to the participant, to be completed before their study visit.

4. Persons will undergo external measurements obtaining weight, height, arm volumetric measurements (Perometer), skin thickness (SkinFibroMeter), and extracellular fluid levels in the arms (InBODY 770, L-Dex and MoistureMeterD/LymphScanner) and upper body (InBODY 770, MoistureMeterD/LymphScanner) and then undergo a non-invasive MRI to evaluate lymph fluid properties in the upper extremities. No MR methods used require the administration of any exogenous contrast agents, and therefore will be completely non-invasive. The visit for non-MRI and MRI measures lasts

approximately 90minutes. Patients undergoing 2-year monitoring will also undergo these non-MRI measurements at baseline, and every 5-7 months (targeting at 6months) up to 5 total visits within 2 years; and for this same group an MRI will be done at Visit 1 and then as needed based on symptoms, clinical therapies provided increasing risk of lymphedema such as radiation, and non-MRI measures at subsequent follow-up visits within the 2 year time period. For these follow-up visits non-involving MRI, the visits last approximately 30 minutes.

Perometer

L-Dex

LymphaTouch

MoistureMeter D/LymphaScanner

InBODY 770 (Body Composition)

SkinFibroMeter

5. For subjects enrolled in Aim 3 of this study, they will receive the above mentioned non-MRI and MRI measures (#2) at Visits 1, 11, 12 and 22. In addition the MLD screening form will be completed on Visit 1 in preparation for this component of their CDT during subsequent treatment visits, as well as completion of the Functional Outcome Measures (PSFS and UEFI) patient self-reports will be done on Visits 1, 11, 12 and 22 to establish baseline functional level and monitor during treatments. Then they will receive their assigned therapy intervention (CDT alone or CDT with graded negative pressure) at Visits 2-10 and 13-21. For Group 1, the first course of treatment will be CDT alone, then the second course of treatment will be CDT with graded negative pressure. For Group 2, the first course of treatment will be CDT alone, then the second course of treatment will be CDT with graded negative pressure. During all courses of treatment, it will consist of 9 visits within 6-8 weeks at 75minutes duration each visit, twice per week for three weeks and then once per week for 3-5 weeks. There will be a 4-8 week washout period between Visit 11 and Visit 12.
6. For the subgroup of subjects enrolled in the 1 time hourly treatment visit of Aim 3, they will receive the above mentioned MRI and nonMRI measures before and immediately following the 1x hourly treatment. The PSFS and UEFI questionnaires will not be administered for this 1x treatment visit only. The participants will undergo (i.) CDT alone, (ii.) CDT with graded negative pressure, or (iii.) exercise and self management treatment. This subgroup participation may involve undergoing all three of the single treatment options on different study dates if the participant is interested oterhwise the study only only involves this one-time study visit.
7. Post processing of this MRI data will be overseen by Dr. Manus Donahue and performed by Dr. Manus Donahue or a member of the KSP team. The KSPs analyzing the data in Aim 3 will be blinded of the subjects' group assignments until after data processing and statistically analysis.
8. Images from cases showing examples of findings that correlate with changes in lymphatic structure and function will be de-identified before inclusion in any potential publication or presentation of the results of this pilot study and saved by Dr. Manus Donahue.

9. Compensation will be as follows:
    a. Aim 1 = $60
    b. Aim 2 = $20 (non-MR measures) / $40 (MRI)
    c. Aim 3
        i. Scan 1 for 1st Treatment = Choice of check for $60 OR 1 Velcro day and night-time compression wrap for arm and hand
        ii. If all 9 treatments + exit visit MRI/non-MRI measures complete = $60 iii. After 4-8 month washout period iv. Scan 1 for 2nd Treatment = $60
        v. If all 9 treatments + secondary MRI complete = $60
        vi. If completes all parts of the study, will earn additional $50
        vii. Total = $120+$120+$50 = $290 OR Total = 1 Velcro compression garment (free) + $60 + 120 + $50 = $230 + 1 Velcro compression garment
    d. Subgroup Aim 3 each one-time visit treatment (pre and post MRI and non MRI measures = $80

6.0 Adverse event reporting

Adverse events (AEs) will be reported according to IRB policies and procedures. Reporting will depend on adverse event severity:

Grading of Severity

0. No AE or within normal limits.
1. Mild AE.
2. Moderate AE.
3. Severe AE resulting in inpatient hospitalization, or a persistent or significant disability/incapacity.
4. Life-threatening or disabling AE.
5. Fatal AE.

AE's of grade three or higher will be reported immediately to the IRB using the Report of Adverse Events per IRB policies and procedures. Every six months the PI will summarize any AE's to the IRB. In conjunction with the IRB, the PI will determine if modifications to the protocol are warranted.

Serious and unexpected adverse events will be reported to our IRB within seven calendar days. All our adverse events will be reported as required at the time of continuing review. Protocol deviations and violations will be reported to our IRB within ten working days.

7.0 Study withdrawal/discontinuation

Subjects will be withdrawn from the study if they will not lie still in the scanner during data collection. In addition, subjects can voluntarily withdraw from the study at any time.

8.0 Statistical analysis plan

Statistical analysis will be overseen by statistician co-investigators. Means, standard deviations, and ranges for continuous variables will be recorded, along with tests for normality and homoscedasticity. The primary statistical objective is to assess whether the changes, pre- vs. post-intervention, in internal imaging metrics of lymphatic clearance or PSFS score are different for the standard CDT therapy or CDT therapy with negative pressure. First, a Student's t-test or Wilcoxon signed-rank test (depending on normality results) will be applied to test the difference between each measurement for the same subject for each of the two therapy sessions. We will also test the difference of any potential carryover effect and we will apply a general linear model approach, in which we include the treatment and treatment-period interaction in the model. Power calculations are based on our preliminary data that observed an improvement in PSFS in five patients on CDT with negative pressure vs. CDT alone, as well as an ability to detect a mean change in T_2 and lymphangiography score pre- vs. post-MLD of N. With the premise that CDT with negative pressure will be at least as good as CDT without negative pressure, with the proposed sample size, we will have 80% power to detect a 40% reduction rate with one-sided type I error = 5%.

9.0 Privacy/confidentiality issues

The data files generated by the MRI scanner, Perometer and L-Dex software are coded using a project name and unique number generated sequentially. Only the investigators will have the key to the subject codes. Image analysis will use the coded files. The safety surveys described above include questions about sensitive health issues. This information will remain with the signed consent form in a secured area near the scanner. These records will remain in the investigators' possession for ten years following the termination of the study and will then be destroyed.

Risks to participants are minimized by limiting this prospective study to subjects meeting the inclusion/exclusion criteria. Access to PHI will be limited to the PI and co-investigators. All data will be de-identified before publication and/or presentation of the results of this study. All PHI used in this study will be kept under lock and key in Dr. Paula Donahue's or Dr. Manus Donahue's office. The primary risk in this study involves the disclosure of protected health information. This risk is reasonable, given the above precautions, in relation to the potential benefit to both future patients and society in general by the possibility of better understanding and treating lymphedema.

10.0 Follow-up

No specific follow-up scans are required for Aim 1 unless patients are eligible and would like to enroll in the 2-year monitoring aim of the study (Aim2). For Aim 2, subjects will be able to contact the PI and co-investigators with any questions and a KSP will contact them to schedule their 6-8 month follow up visits for a total of 4 within the 2 year monitoring period. For Aim 3, subjects will be scheduled for all treatment visits prior to start of each course of treatment (Visits 2-10 and 13-21). The subjects will be able to contact the KSP with any questions and needs to change their assigned treatment times and dates should any adjustments be necessary. The subjects will be contacted during their washout period to schedule their second course of treatment visits and intake and exit measurement visits. An unwillingness to participate in any future scans or treatments will not influence or disqualify a volunteer from participating in the first instance. For the one-time treatment subgroup of Aim 3, this is a one-time visit for the pre and post MRI and nonMRI measures with no further study follow-up unless the participant wishes to undergo all three separate treatment interventions at different dates for interperson treatment comparison. For participants in Aim 2 (monitoring period), if the lymphedema specialist recommends lymphedema management, then the subject will be encouraged to seek out clinical care which does not prevent them from being able to participate in this study, including patients being monitored over 2-years. The lymphedema specialist will help the client obtain orders and location of treatment if subject would like assistance.

11.0 Record retention

Image data will be acquired by the 3 Tesla scanner and will be stored both on digital media (which will not leave the secured scanner area) and on the Institute of Imaging Science server, which is protected by institutional firewalls and is password protected. Investigators may archive these data for their own use, in which case they will be kept in the relevant investigator's locked office. Ten years after the study is concluded, the image data will be destroyed.

In compliance with the National Institute of Health data sharing initiative, imaging data without any personal information attached may be shared with other investigators or public data repositories, which provides the research community with open access to datasets contributed by labs around the world. Information will be completely anonymized with demographics limited to age (accurate to the year up to 90 years old, or "90+" for older individuals), gender (male, female), group membership (e.g., disease/treatment state) and handedness. Data will be transferred using secure file transfer protocols.

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