Postoperative Radiotherapy Breast Cancer Treatment: Musculoskeletal and Functional Implications

A Doctoral Dissertation Proposal
SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FUFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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September 28, 2023

ACKNOWLEDGEMENTS

I am extremely thankful for so many people who have supported me on this journey. The PhD was not a route that I was intentionally seeking, but one that posed an opportunity, a symbolic brass ring, that I could not pass up. It opened a doorway through which I hope to make more of a difference in the life of breast cancer survivors. It was a typical journey of perseverance, faith, sweat and tears, of working at all hours sometimes 7 days a week so I could make Chris' baseball tournaments and Tyler's basketball games, so I could be home and present for them while continuing to pursue my own goals. It was a challenging, amazing, frustrating, inspirational balancing act that I will never forget and never regret.

Thank you first of all to my family, my rock. My boys, Chris and Ty, my parents Bob and Judi Braudy, sister Rachel Shea and her husband Joe, nieces Katie and Kylee Shea, and as silly as it may be, my dog, Storm, who has been there happily wagging her tail and wanting hugs every single day without fail. Also thank you to my aunt, Penny Harris and cousin Wendy Lake, who encouraged me and listened to me when I needed support and guidance.

Thank you to my mentors, Dr. Linda Koehler and Dr. Paula Ludewig, who encouraged and supported me along the way. You both held up the carrot and although I resisted for a while, I am so glad I leaped. Dr. Koehler, thank you for supporting me for the first 3 years and giving me the opportunity to work with your AWS research, but even more so, thank you for your support and friendship. Dr. Ludewig, your collaborative laboratory and phenomenal biomechanics expertise as well as your belief in me … I cannot thank you enough. I will forever remember you poking me in the arm saying, "are you going to do the PhD?" and "you can have your PhD in 4 or 5 years, or you can continue to be a successful clinician in the same space" (paraphrased). I had no chance against sound rationale such as that.

A huge thank you to my committee members, without whom I would not have succeeded. Dr. Jianling Yuan encouraged me and showed amazing patience with my many questions as I dove into radiation oncology literature, clinical applications, and research. Dr. Andrew Hansen believed in my research and writing potential, which were priceless when imposter syndrome invariably hit. Dr. Dawn Lowe, as both a committee member and Director of Graduate Studies, was a solid and valued mentor and I valued our open and closed-door conversations immensely.

Many at the UofM were integral in my journey and I am forever thankful for the help they offered: Shane Edlund, for his willingness to help over and above what his job required as well for his radiation oncology expertise and encouragement, Halley Winters and Kerry Norgaard for their omnipresent smiles (and chocolate kisses) and willingness to help keep me organized and sane, Conrad Lundquist for his humor and problem-solving abilities, Rich Adamczak for always keep me on schedule, and Dr. Rick Jansen for his amazing patience and statistical skills. There are many others as well.

Thank you to all of my friends, especially my girlfriends, who were my rock and held me high not just through the PhD, but through a divorce, moving into a new home, and COVID at the same time. You all are priceless, and I could never have done it without you. It takes a village, and you are all forever a piece of mine. I only hope I can play as supportive a role in your journeys as you all did for me.

Thank you also to JRS, my rock, my partner. What a journey it has been so far, and I cannot wait to experience the rest with you by my side. What a blessing that is.

I hope I am a role model for my children, an example of hard work and self-confidence. An example how to believe in yourself and push yourself to be the best even when some do not believe in you. I want my boys to learn to learn that it is possible to work hard and be successful, to love what you do and the importance of helping others. I want them to learn that the 'work-life' balance is possible, but never to forget that family and friends and connection are paramount. I also want them to know that their mom is strong and capable and will love them to the moon and back forever.

A few quotes that sustained me during these 4 years, and a few more to inspire me or others moving forward (my boys know how I love my inspirational quotes):
"Keep on swimming" – Dory (Pixar)
"Keep your eye on the prize" – Pete Seeger
"Success is liking yourself, liking what you do, and liking how you do it." – Maya Angelou
"Don't just aspire to make a living. Aspire to make a difference." – Denzel Washington

Here's to continuing to learn, give back, tikkun olam (repairing the world), appreciating all the little moments along the way, and in the infamous words of Forrest Gump, "Mama always said: life is like a box of chocolates. You never know what you're gonna get". I can't wait to find out.

ABSTRACT

Purpose/Hypotheses: The overall purpose of this study was to better understand the effect of post-lumpectomy radiation therapy (RT) on skeletal muscle morphology, shoulder kinematics, and shoulder function following treatment for unilateral breast cancer. We hypothesized that within the same breast cancer survivor, the affected (treated) side would demonstrate significantly different shoulder kinematics and skeletal muscle morphology than the unaffected (untreated) side. We also hypothesized that RT dose delivered to specific muscles within the radiation field would adversely affect self-reported shoulder function. A small study was first performed on healthy volunteers to determine intra-rater reliability of a novel method of skeletal muscle B-mode ultrasonography (US) to evaluate echo intensity (EI) and cross-sectional area (CSA) of three muscles within the radiation field that have the potential to affect shoulder function.

Number of Participants: 31 (5 healthy volunteers for US reliability, 26 breast cancer survivors for main study)

Materials and Methods: This was a single center, non-therapeutic, observational cross-sectional study with two parts. First, 5 healthy volunteers participated in the US reliability study which involved three repeated measures of the pectoralis major (PMaj), pectoralis minor (PMin), and serratus anterior (SA) bilaterally. Second, 26 breast cancer survivors who were at least 1-year post-completion of RT following lumpectomy plus sentinel lymph node biopsy for the treatment of unilateral breast cancer then participated in the main study. Three-dimensional kinematic data were collected using electromagnetic sensors during forward shoulder flexion and abduction. Musculoskeletal US was used to determine skeletal muscle CSA and EI of the PMaj, PMin, and SA muscles of the treated and untreated sides. Radiation dose analyses were performed for those same 3 muscles using pre-existing computed tomography radiation simulation scans. The Penn Shoulder Score (PSS) and a custom questionnaire were also given to participants. Data were analyzed using Wilcoxon rank sum tests to determine difference across sides and groups, Spearman correlation to examine associations between variables, and multiple linear regression to examine covariate effects. Ultrasound intrarater reliability was performed on the healthy participants using intraclass correlation coefficient (ICC) analysis. Statistical significance cutoff value was set at 0.05 for all tests.

Results: PMaj and PMin CSA and EI were reliable (ICC > 0.70) and used in the breast cancer survivor study. SA CSA and EI were not reliable (ICC < 0.7) and were used in the main study as exploratory analyses only. Breast cancer survivors demonstrated more sternoclavicular elevation during arm elevation on their affected side vs. their unaffected side. No significant differences

existed between the affected and unaffected sides for other shoulder kinematic variables nor for ultrasound EI and CSA. In general, Penn Shoulder Score values were high, but a few specific functional movements were more commonly noted as being difficult which has clinical implications. Some PMin, PMaj, and SA radiation values were significantly correlated with multiple aspects of the PSS (total score and subscales). Trends were found for the PMin radiation dose and total radiation dose to affect the PSS, although correction for multiple testing made these statistically insignificant.

Conclusions: Our data suggests that there may be a significant effect of postoperative RT on shoulder function in breast cancer survivors after unilateral lumpectomy and sentinel lymph node biopsy. Kinematic analysis demonstrated increased clavicle elevation on the affected side vs the unaffected side during arm elevation, but clinical relevance is uncertain. B-mode US was a reliable method of quantifying PMaj and PMin CSA and EI, but it was not reliable for the SA. B-mode US may not be sensitive enough to detect significant differences in EI and CSA in these muscles following RT. The PMaj, PMin, and SA receive a significant amount of radiation during treatment which may affect patient-reported shoulder pain. Although PSS scores were generally high, participants consistently reported 'some difficulty' with certain functional tasks that highlight the specific impairments many breast cancer survivors have following treatment. Additionally, breast cancer survivors complained not just of 'shoulder pain' but also stiffness, tightness, achiness, and other impairments in their shoulder, chest wall, and arm that need to be recognized and addressed by medical providers. This research demonstrates potential relationships between adjuvant RT and shoulder function which need to be further investigated to provide breast cancer survivors with the highest quality of life possible.

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LIST OF ABBREVIATIONS

Acronym	Definition
AC	**acromioclavicular**
3D	**three-dimensional**
3D CRT	**three-dimensional conformal radiation therapy**
AE	**adverse event**
ALND	**axillary lymph node dissection**
AWS	**axillary web syndrome**
BCS	**breast conservation surgery**
BCT	**breast conservation therapy**
CSA	**cross sectional area**
CT	**computer tomography**
CTCAE	**Common Terminology Criteria for Adverse Events**
DVH	**dose volume histogram**
EI	**echo intensity**
FoB	**Flock of Birds**
GH	**glenohumeral**
GCP	**Good Clinical Practice**
GY	**gray**
IMRT	**intensity modulated radiation therapy**
IRB	**Institutional Review Board**
MCC	**Masonic Cancer Center**
MSKUS	**musculoskeletal ultrasound**
PI	**Principal Investigator**
PSS	**Penn Shoulder Score**
PT	**physical therapy**
QOL	**quality of life**
RLNR	**region lymph node radiation**
ROI	**region of interest**
ROM	**range of motion**
RT	**radiation therapy**
SC	**sternoclavicular**
SLNB	**sentinel lymph node biopsy**
ST	**scapulothoracic**
UMN	**University of Minnesota**
US	**Ultrasound**
WBI	**whole breast irradiation**

Chapter 1: Introduction

Background, Significance and Purpose

In 2022, over 4 million women were living with a history of breast cancer in the United States.(1) Currently, the survival rate of early stage (localized) breast cancers is 99.0% and overall 5-year survival is 90.3%.(2) As detection and treatment will continue to improve over time, the number of breast cancer survivors will increase. The need for addressing sequelae stemming from the cancer itself or from its treatments such as shoulder dysfunction, weakness, pain, fatigue, and difficulty with activities of daily living (ADLs) will also increase. Comprehensive medical cancer care should continue even after active cancer treatment ends. It is our responsibility as multidisciplinary medical providers to help our patients achieve optimal levels of independence, function, and quality of life both during and following breast cancer treatment.

Shoulder dysfunction is one common side effect of breast cancer treatment that has the potential to negatively impact health related quality of life by adversely affecting shoulder range of motion, strength, pain, function, independence with ADLs, and return to work**.** (3–17) Up to 70% of breast cancer survivors have difficulty with shoulder function after treatment (18) and up to 91% of breast cancer patients who receive radiation therapy (RT) have difficulty with upper extremity function up to 5 years after the completion of RT (9). The American Cancer Society / American Society of Clinical Oncology Breast Survivorship Guideline (2016) states that breast cancer survivors complain of limited shoulder range of motion (ROM)(1.5% - 50%), musculoskeletal pain (12% - 51%), upper extremity weakness (18% - 23%) and numbness (29% - 81%).(19) Long term shoulder morbidity is common, as 49% of women have functional impairments, 64% have pain, swelling, or decreased shoulder mobility, and 3% - 25% have severely reduced shoulder mobility more than 10 years after surgical treatment for breast cancer.(6) However, we do not yet fully understand why shoulder dysfunction is so common in this population.

Breast cancer treatment consists of a variety of interventions including local treatments (breast surgery with or without reconstruction, lymph node surgery, and radiation therapy (RT)) as well as systemic treatments (chemotherapy, hormonal therapy, immunotherapy, and targeted therapies). Each treatment type has unique side effects due to its highly specific therapeutic approaches. Radiation therapy is commonly used to treat early-stage breast cancer as it has been shown to decrease locoregional failure, recurrence, and breast cancer mortality. (20)(21,22) The use of RT

may continue to rise, as there is a trend towards breast conservation surgery (i.e., lumpectomy) and RT over mastectomy for control of early-stage breast cancer due to equivalent survival rates with fewer surgical complications and less postoperative pain. (17,21) Axillary RT is also the preferred treatment compared to axillary lymph node dissection (ALND) for patients with early stage (T1-T2) breast cancer and no palpable lymph node disease. (23,24)

Radiation therapy has been shown to increase the risk of shoulder and arm morbidity more than 6-fold. (8,12,12,25–34) Although the exact mechanism by which this occurs is not well understood, it is thought that this may be due to increased soft tissue fibrosis, vascular damage, or tissue atrophy within the radiation field. (3,32,35–39) Recent research has shown that RT affects not only the targeted tissues, but also healthy tissues within the radiation field including those related to shoulder function. (9,25,26,40–42) Current American Society for Radiation Oncology guidelines recommend minimizing dose to normal tissues to minimize such damage. (43) However, these recommendations have historically considered only the heart and lungs as organs at risk (OARs) and have not taken into consideration other adjacent structures including muscles, nerves, and other soft tissues within the radiation field**.** By current standards, the traditional radiation field that includes the breast and chest wall also irradiates the ventral side of the pectoralis major (PMaj) muscle and the ribs as well as the pectoralis minor (PMin) and part of the serratus anterior (SA) due to their anatomic locations on the chest wall. (44,45) As normal healthy upper extremity function is a complex and intricate function of the skeletal, muscular, integumentary, and nervous systems, treatment-induced physiological damage to any of these systems has the potential to affect local tissue health, shoulder kinematics, and upper extremity function.

To best understand how upper extremity function is affected by breast cancer treatment, and more specifically by adjuvant RT, it is imperative to examine shoulder function from a few perspectives. Three-dimensional (3D) kinematic analysis can be used to study shoulder joint movement (46–48). Ultrasound (US) can be used to examine skeletal muscle morphology, i.e., size and quality, in the radiation field, (49–51). Computed tomography (CT) scans that are used to plan radiation treatment can be re-evaluated to learn how much radiation was also delivered to muscles within the radiation field (52). Patient-reported outcomes can be used to better understand shoulder function as experienced by the patients themselves.(53) Although previous breast cancer survivor research has examined these methodologies individually, to the best of our knowledge no study has included these 4 components together: skeletal muscle radiation dose, skeletal muscle morphology (size and quality), 3D shoulder kinematics, and patient-reported function.

Therefore, the overall purpose of this study was to evaluate the effect of post-lumpectomy RT on shoulder function in breast cancer survivors using a variety of methodologies with the goal of better understanding the high prevalence of shoulder dysfunction in breast cancer survivors. The study was conducted in two sequential parts. The purpose of the first study was to determine feasibility and intra-rater reliability of musculoskeletal ultrasound to examine skeletal muscle morphology (echogenicity (EI) and cross-sectional area (CSA)) of the PMaj, PMin, and SA muscles in healthy individuals. The purpose of the second study was to better understand the effects of adjuvant breast cancer RT on upper extremity function and muscle physiology using 3D shoulder kinematics, US analysis of skeletal muscle morphology, skeletal muscle RT dose analysis, and patient-reported shoulder function in breast cancer survivors more than 1 year after the completion of post-lumpectomy radiotherapy.

A better understanding of how adjuvant RT affects muscle physiology and shoulder function can contribute to early detection of, and proactive treatment for, shoulder dysfunction in breast cancer survivors. Shoulder and upper quadrant rehabilitation programs could be designed to address needs of breast cancer survivors both during and after adjuvant RT. This method of study, RT dose analysis with correlation to functional outcomes, could also be applied to other cancer types such as head and neck cancers, which also involve surgery and radiation therapy with known functional deficits following treatment.(54) Comprehensive, multidisciplinary patient-centered oncology treatment plans need to address long-term functional sequelae of cancer treatment to truly optimize not just survival, but quality of life.

Aims and Hypotheses

Specific Aim 1 (3D Kinematics):

Determine the effects of postsurgical radiation therapy on 3D shoulder kinematics in breast cancer survivors more than 1 year after the completion of external beam radiation therapy.

Hypothesis 1.1: Scapular upward rotation will be decreased on the postsurgical, irradiated side as compared to the non-surgical, non-irradiated side during forward flexion and abduction as measured by Flock of Birds 3D kinematic analysis.

Hypothesis 1.2: Posterior tilt will be decreased on the postsurgical, irradiated side as compared to the non-surgical, non-irradiated side during forward flexion and abduction as measured by Flock of Birds 3D kinematic analysis.

Specific Aim 2 (Skeletal muscle morphology):

To determine morphologic characteristics (fibrosis and cross-sectional area) of postsurgical radiation treatment on serratus anterior, pectoralis major, and pectoralis minor muscles more than 1 year after the completion of postsurgical radiation therapy in breast cancer survivors using ultrasound.

Hypothesis 2.1: The serratus anterior, pectoralis major, and pectoralis minor of the radiated side will demonstrate increased echo intensity suggestive of increased intramuscular fibrosis as compared to the non-radiated side.

Hypothesis 2.2: The serratus anterior, pectoralis major, and pectoralis minor cross-sectional area of the radiated side will be less than that of the non-radiated side.

Specific Aim 3 (Radiation and Patient-Reported Outcomes):

To determine the relationship between patient reported functional outcomes (Penn Shoulder Score (PSS)) and predicted absorbed radiation dose to, and echogenicity of, the serratus anterior, pectoralis major and pectoralis minor muscles.

Hypothesis 3.1: Radiation dose (max, mean, V10, V15, V20, V30, or V40) to the pectoralis major, pectoralis minor, and/or serratus anterior will be directly related to patient-reported shoulder function.

Hypothesis 3.2: Mean echogenicity of the pectoralis major, pectoralis minor, and serratus anterior muscles will be strongly correlated ($r>0.50$) with shoulder disability as defined by the PSS.

Specific Aim 4 (US Intra-rater Reliability):

To determine intra-rater reliability of ultrasound echogenicity and cross-sectional measurements of the pectoralis major, pectoralis minor, and serratus anterior bilaterally in a cohort of 5 healthy female volunteers.

Hypothesis 4.1: Echogenicity and CSA scores will be reliable as defined by ICC ≥ 0.70 where the ICC is derived from a two-way ANOVA accounting characterizing within-subject variation across the three muscles. (55)(56)

Chapter 2: Literature Review

Introduction

The following literature review provides a summary of what is known about upper extremity dysfunction (UED) in breast cancer survivors, specifically in relation to postoperative radiation therapy. Three-dimensional kinematic analysis of the shoulder in healthy people and breast cancer survivors will be reviewed. The use of musculoskeletal US to determine skeletal muscle morphology, acute and chronic effects of radiation therapy (RT) treatment, and patient-reported outcomes used to evaluate shoulder function in the breast cancer population will be discussed. The final section will synthesize this information and present possible biomechanical etiologies that explain the effect of adjuvant RT on upper extremity function.

Background: Upper Extremity Dysfunction in Breast Cancer Survivors

Upper extremity dysfunction is a common sequalae of breast cancer treatment. It can last for more than 11 years post-treatment (6) and may include decreased shoulder range of motion (6,13,15,16,21,27,28,57–62) weakness, (4,8,11,15,27,28,58,63–66), pain (6,10,13,16,59,65,67)(68), lymphedema, (6,10,13,16,57,58,61,69), upper extremity and/or chest

wall paresthesia, (10,15,65,69), and rotator cuff disease. (3,70) Possible reasons for these unintentional post-treatment sequelae include but are not limited to scar tissue, fibrosis, chemotherapy-induced peripheral neuropathy, surgical nerve injury, muscle tightness or stiffness, disuse atrophy, deconditioning, intercostal neuralgia, and axillary web syndrome. (3,11,15,32,58,64,71–74)

As breast-cancer related arm and shoulder problems are prevalent and negatively associated with quality of life (QOL) (53,75,76), it is important to understand what variables increase the risk of shoulder dysfunction. Previous research supports that both cancer treatment itself as well as individual patient factors play a role in the development of UED. (7)Treatment factors include breast surgery procedure, (18,30,59,62,64) axillary surgery procedure (5,8,13,31,33,60,65,77–79), chemotherapy(60), RT (27,31,33,42,58), and time since surgery (7,59,64). Upper extremity dysfunction has also been associated with specific patient factors such as lymphedema (30,80), intercostobrachial nerve injury (14), age (30,65,77), hand dominance(4), BMI(59,65) and preoperative ROM (60). It is imperative to identify these and other potential specific risk factors in order to best identify, treat, and potentially minimize, UED in breast cancer survivors.

3D Shoulder Kinematics

Shoulder motion can be studied using 3D kinematic analysis and has the potential to reveal underlying biomechanical dysfunctions that can contribute to shoulder pain.(47,48,81–83) The Flock of Birds (FoB) (Ascension Technology., Inc., Burlington, VT, USA) has been validated as a useful and accurate electromagnetic tool for studying 3D shoulder kinematics.(84–86) with good static accuracy (position 1.8mm, RMS orientation 0.5^0) and static resolution (position 0.5mm at 30.5cm, orientation 0.1^0 at 30.5cm) within a 76.2-cm range with an update rate of 144 measurements per second. . Other equipment such as the Polhemus (Polhemus 3Space Fastrak, Colchester, VT) has also been used to determine accuracy of 3D electromagnetic tracking in shoulder movement.(46) Both measurement types are non-invasive and pose no radiation exposure risk to patients unlike fluoroscopy which is used in some advanced kinematic research. (87). Risk mitigation is of utmost ethical importance when studying an oncologic population whose traditional exams and treatments warrant minimal additional nonessentials exposure and other risks for research purposes.(88)

Karduna et al compared kinematic scapular motion data during 4 independent humerothoracic motions using 2 separate skin-based scapula sensor types and scapular bone pin analyses.(46) The two skin mounting techniques consisted of an electromagnetic sensor secured to the posterior-lateral acromion with double sided tape as per Ludewig and Cook (48) and a tracker method, which consisted of a custom device that lay over the scapular spine. The data was validated against motion of the scapula as detected by a receiver attached to the scapula by 2 during bone pins. Movement error was defined as the difference between the skin based and bone pin-based measurements at any given position. Although differences existed between the 2 skin-based measurements, the authors concluded that both methods were "well suited" for shoulder kinematic analysis below 120° of elevation as angles greater than 120° of elevation had high root mean square errors, up to 25° for scapula external rotation at higher angles. The differences between skin-based and bone pin data were primarily due to skin motion artifact errors.

Hannah et al published a review of electromagnetic tracking system analyses and determined that electromagnetic tracking systems were valid and reliable methods of measuring shoulder motion(88). They found that the acromion method has good to excellent inter-trial (ICC 0.88-0.97) and within-day, inter-session (ICC 0.74-0.94) reliability in both healthy and injured subjects for arm elevation movements, especially during the arm raise (as opposed to lowering) portion of the movement (ICC 0.93-0.98). They also supported the use of a humeral cuff for accurate representation of glenohumeral kinematics during slow movements, with RMS errors less than 8°, which is supported by others with the exception of glenohumeral internal/external rotation due to large errors including an average of 11.40° for glenohumeral rotation at maximum arm flexion.(89,90)

Three non-collinear landmarks are necessary for construction of a local coordinate system for each bone (humerus, scapula, clavicle, and thorax). However, the humerus has only 2 non-collinear landmarks, the medial and lateral epicondyles, leaving the center of rotation of the humeral head to be estimated. Meskers et al developed a least-squares spherical method in which they utilized data points on cadaver scapulae, glenoid, labrum, and humeral heads to develop a center of glenohumeral joint rotation, represented by the center of the new sphere developed from those data points(91). The root mean square error (agreement between the measured and reconstructed glenohumeral centers of rotation) was excellent, with 2.32mm for the *x*-coordinate, 2.68mm for the *y*-coordinate, and 3.04mm for the *z*-coordinate.

The clavicle also only has 2 non-collinear landmarks. However, previous research has demonstrated effective methods of tracking clavicular rotations using the thorax' z-axis to assist with building of the clavicular coordinate system, and this was utilized in post processing as part of our data analysis(92). Assessment of clavicle motion using surface sensors has high reliability, with ICCs ranging from 0.93-0.99 and SEMs ranging from 0.9 – 1.8° in a group of healthy volunteers with and without shoulder dysfunction(92).

3D Kinematic Shoulder Analysis in Healthy, Pain-Free Subjects

Normal, healthy shoulder joint motion is a complex and intricate function of glenohumeral (GH), acromioclavicular (AC), sternoclavicular (SC), and scapulothoracic (ST) joint 3D kinematics(46,47,82,93,94). See Figures 1 and 2 below. Scapular kinematics of an arm raise task (flexion, abduction, scapular plane abduction) in healthy individuals includes SC elevation, retraction, and posterior axial rotation as well as AC internal rotation, upward rotation, and posterior tilting(47,94–96). Scapulothoracic motion is a function of combined motions of the AC and SC joints called coupling with different components contributing to scapular upward rotation within distinct ranges of shoulder elevation(97). See Figures 1 and 2. Matsuki et al (2011) found that hand dominance affects scapulohumeral kinematics, with the dominant scapula starting more downwardly rotated than the nondominant scapula, but then moving more rapidly into scapula upward rotation during arm elevation. (98)

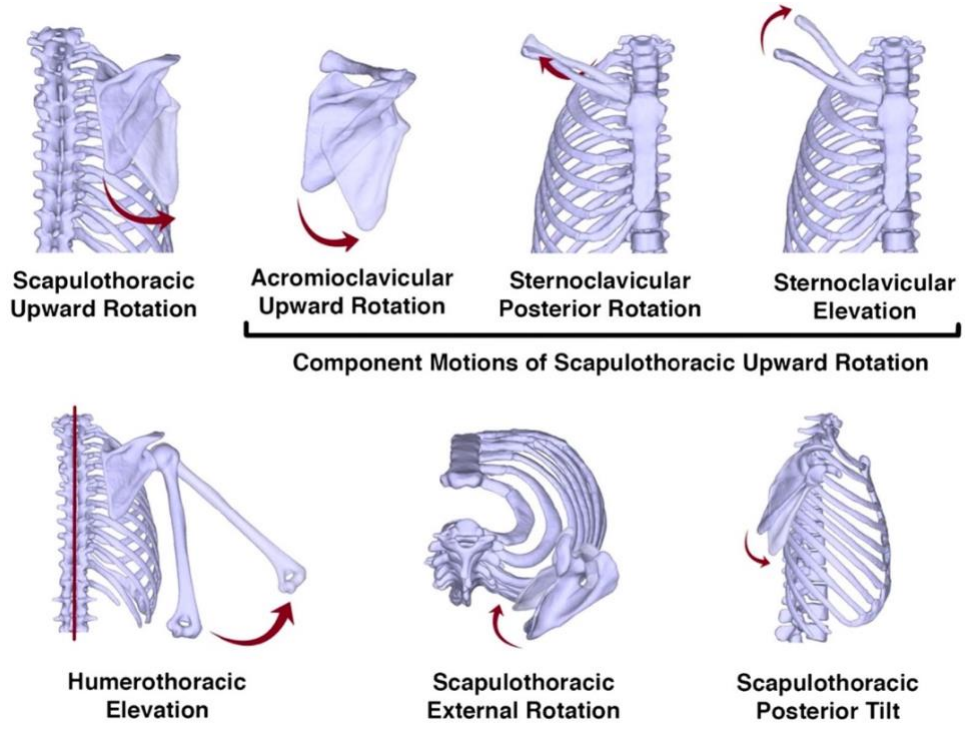

Figure 1. Shoulder motion expressed as component scapulothoracic, acromioclavicular, sternoclavicular, and humerothoracic parts. From Lawrence et al 2020, with permission.(97)

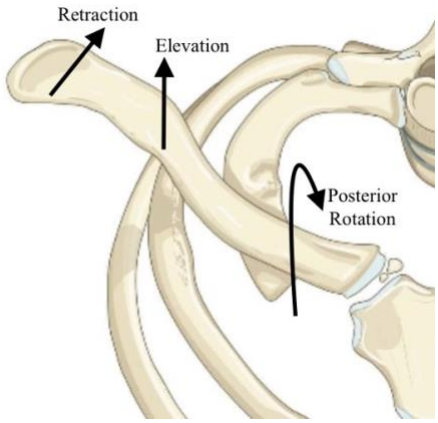

Figure 2. Sternoclavicular joint retraction, elevation, and posterior rotation. (Imaios,with permission) (99)

Quantification of normal movement is necessary to best understand what is considered abnormal. McClure et al used steel bone pins drilled directly into the scapula of healthy volunteers to acquire scapular position and orientation information (100). They found that during 2 planes of arm elevation, the scapula upwardly rotated 46^0 - 50^0, posteriorly tilted 30^0 - 31^0, and externally rotated

24^0 - 26^0. Ludewig et al reported clavicular elevation (11°-15°), retraction (15°-29°), and posterior long-axis rotation (15°-31°) during arm elevation in asymptomatic subjects (92).

Joint mobility is controlled by the pull of muscles and ligaments that attach to those joints as well as by neuromuscular activation of those structures. It can also be influenced by pliability of the soft tissues surrounding joints such as skin and fascia (101). Increased resistance to movement due to tight antagonistic tissues including but not limited to skin, ligaments, and fascia has the potential to increase the workload of the agonist muscle groups and even decrease range of motion or cause compensatory movement patterns in an effort to avoid the increased resistance (3,102,103).

In healthy subjects, the prime movers of the scapula into upward rotation include the serratus anterior (especially the lower serratus anterior) (48,93,101,104,105) and the lower trapezius as well as the middle trapezius (48,101,106). Posterior tilt is controlled primarily by the serratus anterior but also the lower trapezius (48,104,107). Tight anterior structures such as the pectoralis minor have the potential to limit posterior tilt (108). Scapulothoracic external rotation is also primarily controlled by the lower trapezius and serratus anterior as well as the rhomboid major and middle trapezius. Altered muscle activation, especially decreased serratus anterior activation, has been found in those with shoulder pain (81). It is important to highlight that many essential ST joint movements are at least partially controlled by the serratus anterior. Weakness or fatigue of the serratus anterior and other muscles that contribute to scapular motion could affect shoulder kinematics and increase the risk of shoulder pain.

3D Kinematics in Healthy Subjects with Shoulder Pain

Abnormal ST kinematics, or the movement of the scapula on the thorax, has traditionally been thought to be at least partially responsible for shoulder pain in healthy individuals (48,81,82,96). Recently, the contribution of ST kinematics to shoulder pain is being further examined as the data is sometimes contradictory and ST kinematics can vary between studies, subject populations, and with arm elevation angle and plane of elevation (97,109)(110). However, until we find other noninvasive, accurate indices of shoulder kinematics that better explains shoulder dysfunction and / or pain, there is sufficient evidence to continue to explore the relationship between scapular kinematics and shoulder function.

Scapular upward rotation is often decreased in healthy people with shoulder pain (47,48,93) and has been associated with pathologies such as shoulder impingement, rotator cuff tendinopathy, rotator cuff tears, shoulder instability, and adhesive capsulitis (47,48,81,96). A recent review of the literature revealed that the effect of decreased ST upward rotation on subacromial space (one measure of shoulder impingement) varies with arm elevation angle(110). Those with shoulder pain have also shown less SC posterior rotation and elevation (47,48).

Lawrence et al. used bone pins to compare ST kinematics in people with and without shoulder pain (47). They demonstrated that participants with shoulder pain had significantly less scapular upward rotation at 30^0 and 60^0 of arm elevation, less SC posterior rotation throughout the arm raise task, regardless of angle, phase, or plane of shoulder motion, and less SC elevation at 30^0 of arm elevation compared to subjects without shoulder pain. Ludewig and Cook used a non-invasive 3D tracking system (Polhemus®) to determine scapular kinematics in a group of construction workers with and without shoulder pain (48). They found that workers with shoulder pain had decreased scapular upward rotation, increased scapular anterior tipping, and increased scapular medial rotation at various points in the arm elevation cycle and when carrying a 4.6kg load.

In contrast, other studies have found increased scapular upward rotation with arm elevation in patients with shoulder pain. McClure et al discovered that those with shoulder impingement had greater ST upward rotation and clavicular elevation with shoulder flexion and slightly greater ST post tilt and clavicular retraction and greater post tilt during scapular plane abduction (82). The difference in findings may be due to subject selection (subacromial impingement tests and inclusion of both genders with a diverse history of shoulder pain), testing procedures (use of different scapular sensors), compensatory movement patterns, and diverse kinematic patterns present in the general population with shoulder pain as compared to those selected for certain studies.

3D Kinematics in Breast Cancer Survivors

Similar to shoulder kinematic variability seen in healthy subjects, scapular kinematic patterns in breast cancer patients differ depending on the study, likely due to the inclusion of different surgeries, radiation treatment regimens, data collection timepoints, kinematic measurement methods, etc. In addition, only in the last 10-12 years have there been studies published that utilize objective 3D electromagnetic tracking technology to examine shoulder mobility after breast cancer surgery (102,103,111–114). A recent review by Miguel-Andres reviewed 20 articles that examined

shoulder kinematics following mastectomy. They concluded that a variety of kinematic methodologies (optoelectronic and electromagnetic systems), movements studied (functional tasks and planar movements), and surgical interventions (mastectomy, reconstruction, axillary node surgies) made it difficult to compare results (115).

Spinelli et al studied women after mastectomy and immediate breast reconstruction as well as after lumpectomy and whole breast irradiation, an average of 2.5 years after surgery(103). They found that the overall shoulder kinematic patterns of both patient groups mirrored that of healthy individuals, with the scapula upward rotating and posterior tilting with concurrent clavicle elevation and retraction during arm raising and hair combing tasks. However, they also found no significant differences in ST ROM between women with breast cancer and healthy controls, although Penn Scale pain scores were correlated with ST upward rotation during reaching ($r = 0.36$, $p < 0.05$).

Decreased scapular upward rotation has been detected in breast cancer patients who had shoulder pain compared to survivors without shoulder pain and healthy controls (114) and after breast cancer surgery in general, without surgery type defined (116). In one recent study, scapular upward rotation was decreased 15.2° during arm elevation in a population of women with breast cancer with axillary web syndrome as compared to a cohort without axillary web syndrome 5 years after surgery (71).

In contrast, scapular upward rotation has been shown to increase after mastectomy as compared to healthy controls (111), post-lumpectomy (117), and as compared to the uninvolved side (102,113). Another group found scapula alata, or winged scapula, with prevalence ranging from 10.9% of patients 7 weeks postoperatively to 27.7% at 416 days postoperatively (118). This condition often reflects significant weakness or palsy of the serratus anterior and is a known cause of shoulder dysfunction (119). Borstad and Szucs demonstrated increased values of ST internal rotaton by 12.1° at 2 months after surgery as compared to before surgery ($F=16.11$, $p<0.0001$), although surgery type was not specified (102).

Together, these contrasting findings highlight the need for comparisons among similar treatment regimens as different oncologic and plastic surgeries, radiation treatments, chemotherapies and hormone therapy treatments may lead to diverse physiological and functional outcomes. It is obvious and well accepted that one cannot compare outcomes of different orthopaedic shoulder

surgeries (rotator cuff repair, shoulder joint replacement, biceps tenotomy, etc.) Similarly, the outcomes of each breast cancer intervention, whether primary surgical (mastectomy, lumpectomy, ALND, SNB), or reconstruction related (prepectoral vs. subpectoral expander to implant reconstruction, flap surgeries, etc.), cannot be equitably compared either. In addition, the broad categorization of inclusion/exclusion of radiation therapy may yield different outcomes if the extent of radiation (total dose, daily dose, max dose, treatment volume, etc) is examined thoroughly (52).

Musculoskeletal Ultrasound

Ultrasound is a tool which utilizes sound waves to visualize structural heterogeneity in vivo (120) to determine muscle structural changes and detect muscle pathology (49,121). Previous research has validated quantitative muscle ultrasound as a reliable method to determine muscle structural changes, including the presence of fibrosis (122). Ultrasound has traditionally been used to diagnose neuromuscular diseases such as muscular dystrophy (123), but others have used it to examine radiation-induced effects on cardiac muscle (124) and skin (125,126). The same principles can be applied to examine postsurgical RT-induced musculoskeletal physiology and pathology as well (51,127).

Musculoskeletal ultrasound is a less expensive method of tissue analysis than traditional orthopaedic magnetic resonance imaging and computed tomography. Ultrasound is portable, easy to access, and does not expose the subject to radiation as with computed tomography imaging. In ultrasound, a transducer uses the quartz crystals and the piezoelectric effect to transform electrical current into sound waves, which are delivered to the tissue(s) under examination (128). The frequency of the sound wave determines the depth of the examination and is correlated inversely with image resolution (128). Lower frequencies such as 5MHz allow for imaging deeper structures such as muscle and nerve while higher frequencies such as 17MHz allow for the imaging of superficial structures such as skin to determine skin thickness (120,129). Typically, musculoskeletal ultrasound utilizes 5MHz or 7.5MHz transducers (129).

Sound wave reflection is a function of acoustical impedance, which is a combination of the ability of the sound wave to travel through a tissue and the density of the tissue itself (129). Acoustical impedance varies by tissue type: sound velocity of bone is 300 m/s, air 4000 m/s, and muscle approximately 1580 m/s (129). As the sound waves hit tissues of different acoustical impedance, some are reflected back to the transducer head. These sound waves are transformed back into an

electric current (128), after which specific computer programs transform the temporal and acoustic raw data into 2-dimensional images based on the time it takes the signal to be received after being sent (temporality) and the reflected soundwave amplitude (129). The various structures in the image are then differentiated by being assigned a grey scale brightness value otherwise known as echogenicity, which permits structural differentiation (120,129).

The quantity of reflected echoes per square area determines the brightness of the image, or echo intensity (EI). Hyperechoic structures such as bone and tendon reflect much of the sound wave signal and appear white. Hypoechoic tissues such as muscle fiber bundles reflect a small amount of signal and appear darker. Anechoic structures such as fluid and subcutaneous fat appear darkest and do not reflect much signal. Healthy muscle typically looks black in ultrasound due to its low echogenicity, with white hyperechoic lines shown within the muscle and on its perimeter due to the presence of connective tissues that reflect more signal than the muscle fibers themselves (128).

Hernandez-Belmonte et al examined the reliability and validity of panoramic US to detect PMaj CSA (130). They examined reliability of two repeated measurements by a trained and novice operator as well as analysis reliability by determining CSA of the same measurement two times. They also compared experienced versus novice validity by comparing CSA obtained by US to that obtained by MRI. They found that errors were significantly less in image acquisition when performed by the trained ultrasonographer (standard error of the mean = 0.25 cm^2 vs 0.66 cm^2 for trained and novice operator respectively) and analysis (standard error of the mean = 0.27 cm^2 vs 1.13 cm^2 for trained and novice operator respectively). The experienced operator also had smaller errors as compared to MRI than the novice operator (-0.19 +/- 0.34 cm^2 vs -1.97 +/- 2.59 cm^2). They concluded that panoramic US is a reliable and valid technique for measuring PMaj CSA.

Rosenberg et al demonstrated good reliability of simultaneous assessment of muscle size (CSA) and quality (EI) using panoramic US (131). The authors examined the medial gastrocnemius muscle with the patient in prone with the leg stabilized by a Velcro strap to prevent movement and ensure consistent positioning. The distance chosen for examination was 30% of the lower limb length, and transverse US imaging using B-mode and a 5-13 MHz probe with consistent gain, depth, and frequency were utilized for each subject. Echogenicity and CSA data analysis were both performed in ImageJ software (NIH, Bethesda, MD). Reliability ICC and SEM% were 0.914 and 5.830% for CSA and 0.720 and 3.680% for echo intensity. Tanaka et al performed a similar study

examining simultaneous assessment of muscle CSA and echo intensity for abdominal skeletal muscles in 27 young healthy males. They also found high ICC and SEM values for CSA (ICC 0.944 – 0.958 and SEM 4.9% - 7.3%) and for echo intensity (ICC 0.851 – 0.945 and SEM 5.3% - 9.7% respectively) (132).

Echo Intensity / Echogenicity

Musculoskeletal ultrasound (MSKUS) echogenicity values can be used to estimate the amount of fibrous tissue in muscle using grayscale analysis as increased collagen content in post-radiation fibrous tissues increases echogenicity values (51,122,124,126,133–136). In general, healthy muscle appears heterogenous, with muscle fiber bundles appearing hypoechoic and interspersed connective tissue appearing hyperechoic(129). Diseased muscle may appear hyperechoic reflecting an increase in fibrosis or fatty tissues (See Figure 3) which can also be thought of as a relative increase in noncontractile tissues(137).

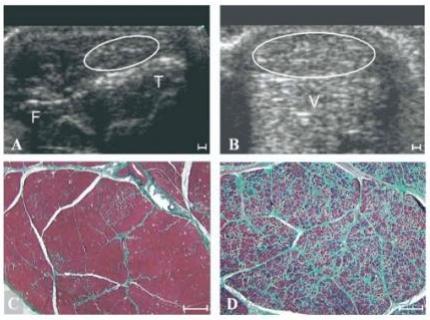

Figure 3. Comparison between musculoskeletal ultrasound imaging of the tibialis anterior echo intensity (A) and rectus femoris (B) and associated muscle histologies (C and D, respectively) in dogs with muscular dystrophy. Image B demonstrates increased echogenicity associated with increased fibrous tissue also observed in image D. From Pillen&Tak2009 (121).

In a study examining the effect of RT on collagen metabolism on breast skin 4 months after breast cancer RT, the synthesis of procollagen was increased 7.7-fold and remained elevated for 2 years. This suggests both early and chronic changes in collagen synthesis (136) which can be measured using US EI techniques. In fact, in a separate study, ultrasound B-mode was used to demonstrate 11.6% increase in echo intensity ($p = 0.002$) of the vaginal wall 1-3 years after treatment suggestive of increased fibrosis in a population of women treated with surgery plus RT receiving treatment for gynecologic malignancy as compared to women treated with surgery alone (51).

In a population of 20 healthy females, US-derived skeletal muscle measurements (muscle thickness and EI) were independently associated with lean body mass ($r = 0.64$, $p = 0.002$). Additionally, EI of the rectus femoris was significantly associated with strength ($\rho = -0.67$, $p = 0.001$) (Ismail 2005). Additionally, in a population of men 65-91 years of age, ultrasound derived EI of the right thigh was significantly and negatively correlated with muscle strength even after correction for age, weight, height, and fat thickness ($r = -0.333$, $p<0.001$) (138). These two studies further demonstrate clinical relevance of ultrasound-derived EI data as it relates to strength and lean body mass.

Echo intensity values can be compared across subjects or conditions, especially when compared across groups (139). Additionally, intra-rater and inter-rater reliability of MSKUS grayscale analysis using 2 different techniques (Rectangular Marquee Tool and Free Hand Tool) in Photoshop and ImageJ (95%CI ICC = 0.97-.99, $p < 0.001$) and uniform coefficients of determination (R^2 = 0.096-.99, $p < 0.001$) were excellent in a population of 18 men examining their rectus femoris muscle using B-mode ultrasound (134). The Rectangular Marquee Tool allowed for faster area selection, but the free hand tool allowed for more variation in perimeter selection which they stated could be useful for muscles with non-rectangular shapes. The authors concluded that both Photoshop and ImageJ are good methods for tissue EI analysis in older adults.

Cross-sectional Area

Musculoskeletal ultrasound also allows us to quantify muscle thickness using CSA and has been previously found to be a reliable and valid imaging technique (130,140). Cross sectional area allows us to estimate muscle atrophy when compared to pre-treatment conditions, a control, or to standard, healthy norms. Electronic calipers can be utilized to measures muscle CSA defined by calculating the maximal distance between preselected anatomical muscle boundaries, or region of interest (ROI). This measurement has been found to have good reliability and validity with a mean intraclass correlation coefficient of 0.998 for reliability and 0.999 for validity as compared to MRI in large individual human muscles (50). Cross sectional area is an important value as it a strong predictor of maximum voluntary force in the elderly (141).

Very little literature is available regarding the use of US to quantify muscle CSA in oncology populations. Wolfram et al measured sternocostal and clavicular PMaj thickness (not CSA) using US in 6 participants following lumpectomy + SLNB + adjuvant RT to the breast only (42). They found a decrease in sternocostal PMaj mean thickness by 22.6% between 30 days and 6 months

post-RT (Cohen's dz = 1.29) Mean thickness of the clavicular PMaj was less and cited as 5/6 participants having a mean thickness decrease of 12.3% from 6-months to 12-months post-RT (Cohen's dz = 0.86).

Ultrasound B-mode has been used to demonstrate 153.2% increase in vaginal wall thickness following RT as compared to a control group in a population of women treated for gynecological cancers. However, a sample size of 6 receiving RT (and 6 controls) limited the author's ability to subgroup with enough power needed to understand the effect of radiation dose, external vs. internal radiation treatment, and fibrosis severity (51). Most of the literature explores the effect of radiation on skin and subcutaneous tissues (125,142) and the heart (124). The effect of RT on skeletal muscle CSA and the subsequent association with muscle atrophy, strength, and other markers of function and quality of life are not well understood.

In summary, RT has the potential to decrease muscle thickness and increase skeletal muscle fibrosis both of which can be measured using ultrasound. These conditions may have significant negative implications on upper extremity force, function, and independence with daily activities in breast cancer survivors years after completion of surgery and radiation. More information is needed to better understand these relationships both from diagnostic and treatment viewpoints.

Breast Cancer Treatment Overview

The treatment of breast cancer is highly complex and dependent upon each patient's individual cancer type, stage, age, comorbidities, and numerous other factors (143). Local treatments can include breast and lymph node surgeries as well as radiation therapy, sometimes followed by reconstructive therapies. Systemic treatments can vary from none to a combination of the following: chemotherapy (given as neoadjuvant chemotherapy, or prior to surgery, or adjuvant chemotherapy, or given after surgery), hormonal therapy, immunotherapy, and targeted therapies (144).

Breast cancer treatment typically involves treatment of 3 main areas: the breast, lymph nodes, and remainder of the body. The two main types of breast surgery are breast conservation surgery (BCS) and mastectomy. Breast conservation surgery is also commonly called partial mastectomy, segmental mastectomy, lumpectomy, or quadrantectomy. All refer to removal of the tumor from the breast with a clear margin, meaning no cancer cells are located within a predetermined sample

perimeter. Mastectomy is removal of the entire breast and traditionally, the pectoralis fascia. Bilateral mastectomy is the surgical removal of both breasts, with one side being prophylactic in the case of unilateral breast cancer (145,146).

Surgical lymph node treatment consists of either sentinel lymph node biopsy (SLNB), or removal of the first node or nodes that drain the breast with the goal of detecting cancer cells that have left the primary tumor (145), or axillary lymph node dissection (ALND), which is the removal of at least 10 level I and level II lymph nodes (below the inferior portion of the pectoralis minor muscle and underneath the pectoralis minor muscle, respectively) (147). ALND is typically utilized for those with clinically positive lymph nodes. The less extensive SLNB is now the standard of care for those who have clinically negative nodes (143). The remainder of the body is treated with chemotherapy, hormone therapy, and / or other medications as indicated.

According to the American Cancer Society, 50% of women with early stage (Stage I or Stage II) breast cancer will have BCS followed by RT (1). Those with Stage III breast cancer are more likely to have mastectomy followed by chemotherapy (65%). Those with Stage IV breast cancer may have chemotherapy and/or radiation therapy, typically without surgery (60%).

As the focus of oncology care now includes survivorship, more emphasis is being placed on minimizing surgery invasiveness and treatment-related sequelae. In 2014, survival after lumpectomy versus mastectomy (with and without RT) was compared in 132,149 patients. Ten-year breast cancer-specific survival rates were highest in the lumpectomy plus RT group (94%) as compared to the mastectomy only group (90%) and mastectomy plus RT (83%), $p<0.001$ (20). This led to a shift in treatment, with more breast cancer patients with early-stage invasive ductal carcinoma having BCT + RT.

An important trial that reflects a similar trend towards minimizing surgery invasiveness for lymph nodes is the ACOSOG Z0011 phase 3 randomized clinical trial (148). This trial was pivotal in changing standard lymph node surgery from the more invasive ALND to the less invasive SLNB followed by RT in women with T1 or T2 (early stage) invasive breast cancer, no palpable adenopathy, and 1 or 2 positive sentinel lymph nodes. There was no difference in overall 10-year survival (SLNB 86.3% vs. ALND 83.6%), 10-year disease-free survival (80.2% SLNB, 78.2% ALND), and 10-year regional recurrence. As SLNB is not only a less invasive surgery but also

carries decreased risk of shoulder morbidity and lymphedema compared to ALND (33,149), this was an important change focused on multifocal patient outcomes and quality of life.

These trends towards minimizing the invasiveness of oncology treatment including surgery and radiation demonstrate a continuous improvement to preserve not just survival, but also QOL. A better understanding of possible side effects / late sequelae that oncology treatments have on patient health related QOL and function are implicit in improving and advancing oncology care from treatment to survivorship.

Radiation Therapy

Radiation therapy is an essential part of many breast cancer oncology care plans as it has been shown to reduce the risk of locoregional failure, recurrence, and breast cancer mortality while prioritizing breast conservation over the more invasive mastectomy procedure (21,22,150). Traditional external beam RT utilizes photons to damage tissue DNA within the radiation field, causing irreparable harm to both microscopic cancer cells and healthy tissues (150,151). It damages DNA via free radical generation and reactive oxygen intermediate production as well as by a secondary inflammatory response, leading to cancer cell death(150,151).

Radiation Indications / Guidelines

Indications for RT are complex and dependent upon many variables, including tumor size, nodal involvement, presence/absence of metastases, response to preoperative systemic therapy and ability to operate on tumor, surgery type, surgical margins (presence/absence of tumor at a defined periphery of surgical specimen), and other factors (143). The National Comprehensive Cancer Network recently published guidelines for the treatment of breast cancer (see immediately below) (143). If a patient has neoadjuvant chemotherapy (chemotherapy prior to surgery), the radiation plan is based on the disease stage prior to chemotherapy plus the pathology results following chemotherapy. The treatment plan including targeted tissues, total dose given and dose per fraction depends on many factors which are beyond the scope of this dissertation. Treatment plans relevant to this dissertation are described below as described in Macdonald et al (143).

- *Whole breast* radiation includes a hypofractionated dose of 40-42.5 Gy in 15-16 fractions, with occasional use of a more conventional treatment regimen of 45-50.4Gy in 25-28 fractions in some cases. A boost, or extra dose of radiation to the tumor bed, can be used if there is a high risk of recurrence and adds 10-16 Gy in 4-8 fractions.

- R*egional lymph node* radiation can include axillary, supraclavicular, and internal mammary lymph nodes. Typical dose is 45-50.4Gy in 25-28 fractions or a hypofractionated dose of 40-42.5Gy in 15-16 fractions with an additional boost in certain cases.
- *Hypofractionation,* or the use of larger daily fractions and a smaller total dose over a shorter period, is becoming more common as it allows for the potential of a shorter treatment time, less expense to the patient and hospitals, and less skin toxicity (152). This is delivered as 39 – 42.9 Gy in 15-16 fractions of 2.6 – 3.3 Gy (143).

The aim of RT is to maximize the dose delivered to the area at risk to decrease the chance of local recurrence while minimizing exposure to surrounding, healthy tissues (152). The executive summary of the American Society for Radiation Oncology ASTRO guidelines states that "when planning, the volume of breast tissue receiving >105% of the prescription dose should be minimized and the tumor bed contoured with a goal of coverage with at least 95% of the prescription dose. Dose to the heart, contralateral breast, lung, and other normal tissue should be minimized"(43).

This concept of target field optimization and minimization of radiation delivery to OARs such as the heart is the basis for this proposed research as other healthy tissues are unavoidably affected to maximize target dose (153). These other tissues, as first pointed out by Lipps et al, include skeletal muscles of the chest wall that contribute to shoulder function (52).

Radiation Field and Types

The breast cancer radiation field may include the whole breast, partial breast, and/or chest wall as well as axillary, supraclavicular, and/or internal mammary lymph nodes in some advanced cases (35,44,150,154). A standard tangent field typically extends from the inferior edge of the clavicle superiorly to 2cm below the infra-mammary fold or 1-2cm below the lower limit of the breast inferiorly, medially to body midline (more when internal mammary nodes are irradiated), and laterally to the midaxillary line, with optimization of the posterior border to avoid OARs.(45) This standard tangential field often includes level I and part of level II lymph nodes unintentionally, but unavoidably, due to anatomy. Regional lymph nodes are also radiated in cases where lymph nodes are positive, or at the discretion of the radiation oncologist (143). This may include:

- The supraclavicular field is included in addition to tangent fields when lymph nodes are positive (contain cancer cells) or the cancer is staged as T3/4 (45,155). Treatment extends

to 3cm in depth from the inferior border of the cricoid cartilage of the larynx superiorly to the level of the tangential fields inferiorly, medially to the chest midline, and laterally to the coracoid process, medial to the humeral head.

- The anterior axillary field is added to the supraclavicular field when there is "extensive extranodal extension or inadequate axillary nodal dissection or undissected axilla" (45). In this case, the lateral border is extended to the junction of the medial two-thirds and lateral one-third of the humeral head and the humeral head itself is blocked to prevent radiation damage.
- A posterior axillary field may also be added for better coverage of the axillary region, with midline of the clavicle as superiomedial border, 1.5-2cm of the lung as inferiomedial border, humeral head as superiolateral border, skin of the axilla for inferolateral border, and the level of the tangential fields as the inferior border.

A thorough understanding of the radiation field and its contents is important as organ exposure varies by treatment plan (153). The effects of radiation on OARs such as the heart and lungs have been extensively studied and are a standard and essential part of radiation treatment plan evaluation aimed at minimizing RT dose to these vital organs (45,124,153,156,157). Those with left sided breast cancer are at increased risk of cardiac complications secondary to beam orientation and cardiac anatomy and location in proximity to the targeted breast tissues (124,153). Ko et al demonstrated that among those with left-sided breast cancer, OARs receive significantly different doses of RT dependent upon RT technique and beam delivery system (153).

Tuohinen et al used ultrasound to detect RT-induced myocardial changes including increased echogenicity in the left ventricular septum and right ventricular free wall (124). Left ventricular mass increased in those receiving left sided RT as compared to those receiving right sided RT ($p < 0.05$). They concluded that left sided RT resulted in increased myocardial echogenicity, with greater changes seen in those areas that received a higher dose of RT. Inclusion and/or exclusion of OARs must be carefully considered when choosing a treatment regimen, while preserving the targeted dose to desired tissues. Similarly, Lipps et al extended this analysis type to examine specific skeletal muscles at risk within the radiation field (52).

The finely tuned balance of RT risk vs RT benefit is evident as recent research demonstrated that in certain cases or populations, the risk of adding RT to the oncology treatment regimen may

outweigh the benefit. For example, in its 10-year follow-up, CALGB study 9343 revealed that women 70 years and older with hormone receptor-positive early-stage breast cancer had no difference in overall survival and only a modest improvement in locoregional recurrence, from 10% to 2%, with the addition of RT to endocrine therapy after breast conserving surgery (BCS) (158). As a result, some women over 70 years of ago with qualifying tumor status may have endocrine therapy alone, and not additional RT, thus avoiding any long-term RT sequelae. As science progresses and aims to improve not just overall survival, but quality of life, potential adverse effects of radiation on healthy tissues and the effect of these changes on function and quality of life must be considered.

Radiation Therapy Positioning

Patients are typically positioned in supine with one or both arms overhead to prevent exposure to the upper arm by tangential fields, while maximizing exposure to the breast and axilla (45). See Figure 4. To best delineate the post-lumpectomy radiation field, a simulation is performed during which scar and drain sites are marked with radio-opaque wires. A computerized tomography (CT) scan gives the most accurate visualization of the entire chest wall and is performed from the chin superiorly to the lower border of L1 vertebrae, inferiorly in 3-5mm slices (45).

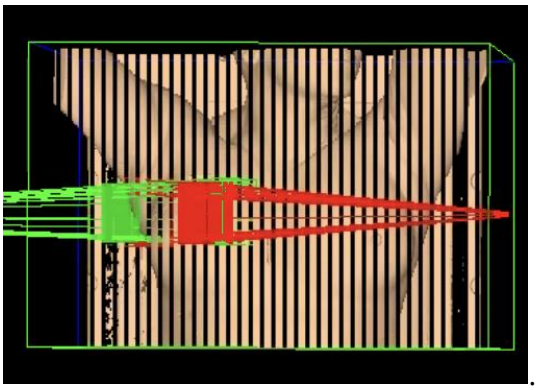

Figure 4. Patient positioning in supine with arms overhead during a computed tomography simulation scan for right breast radiation. The red and green lines represent the RT tangent fields.

Patients are required to have adequate overhead shoulder range of motion for radiation treatment to allow for positioning of the upper extremity outside the radiation field. The exact amount of shoulder range of motion is unknown, but to achieve proximal-mid humerus positioning superior to the clavicle (superior border of tangential field), overhead elevation needs to be greater than 120°, most likely at least 140° of abduction in the scapular plane. Decreased preoperative shoulder

range of motion not only may delay radiation treatment but is also a risk factor for increased shoulder pain following treatment (60).

Trends in Breast Cancer RT

The utilization of lumpectomy plus RT for the management of early stage (Stage I or II) breast cancer increased following a groundbreaking study, NASBP-B06, that compared the hazard ratio for death among 3 randomized treatment groups: total mastectomy, lumpectomy alone, or lumpectomy and breast RT with a 20-year follow-up. The hazard ratio for death among women who had lumpectomy followed by breast RT was 0.97 as compared to those who underwent a total mastectomy. This suggested that survival rate was effectively the same for both treatments, with significantly less surgical intervention needed for the lumpectomy. Additionally, the hazard ratio for death among women who had lumpectomy followed by RT was 0.91 as compared to those who did not have postoperative RT, demonstrating a distinct benefit to the utilization of RT to provide additional protection against death from early stage (Stage I or II) breast cancer (21).

In 2014, the AMAROS trial compared ALND (median of 19 lymph nodes removed) with axillary RT (median of 2 lymph nodes removed) in patients with early stage (T1-T2) primary breast cancer and no palpable lymphadenopathy (24). This multicentered trial randomized 4823 patients to receive ALND (n=2402, with 744 having a positive sentinel node) or axillary RT (n=2404, with 681 having a positive sentinel node.) The primary endpoint was non-inferiority of 5-year axillary recurrence, but this analysis was underpowered as only 4 patients in the ALND group and 7 patients in the axillary RT group had axillary recurrence. Regardless, the authors reported that there were no significant differences between the two groups based on disease free survival and overall survival. Furthermore, axillary RT was found to have significantly less morbidity with less lymphedema noted than in the ALND group, but equivalent shoulder ROM outcomes (24).

Adjuvant RT thus has the potential for long-term adverse effects on shoulder function as it will be included in many treatment programs for those with early-stage breast cancers as well as for those with more advanced breast cancers due to clinically positive nodes. Additionally, more research is needed to understand the impact of radiation on other oncology treatments such as head and neck cancers and ob/gyn cancers as oncology rehabilitation continues to focus on *quality* of survivorship, not just overall survival.

Acute and Late Radiation Effects

Radiation therapy can affect cells in 2 general manners: 1) daily treatment that results in direct damage to DNA secondary to free radical and reactive oxygen intermediate generation, and 2) indirect damage from nearby tissues and vascular immune cell interactions including inflammatory response that can occur months to years later (32,150,151,159). These are often referred to as acute and late effects respectively (159).

Acute Radiation Effects

Acute radiation damage is most common between the 2nd and 4th weeks of treatment in cells that rapidly proliferate such as the skin due to radiation damage to functional cells as well as to stem cells that typically replace those damaged cells (150,159). Radiation causes ionization and free radical production leading to cell death by mitosis or apoptosis (159). It activates proinflammatory and profibrotic cytokines, as well as the coagulation cascade and causes vascular injury which can lead to inflammation, edema, and poor tissue healing.(159,160) Up to 95% of patients receiving RT for the treatment of breast cancer experience acute skin reactions, or radiation dermatitis, including skin erythema, desquamation, and more rarely, tissue necrosis(150). Acute radiation damage occurs within 30 days after RT completion, with peak effects occurring 1-2 weeks after RT completion.

Late Radiation Effects

Late effects of radiation can occur months or years later, and may consist of fibrosis, necrosis, chronic inflammation, atrophy, and / or vascular damage (32,38,150,151,159,161,162). Radiation can damage satellite cells causing decreased activation and proliferation. This can negatively affect muscle health via decreased differentiation of myoblasts and decreased fusion of myocytes that leads to adverse effects on muscle volume and length, and ultimately, shoulder function (32). Late RT effects are most common in tissues with slower turnover than skin such as muscle, subcutaneous tissues, and fatty tissues. Damage to the vasculature can lead to damage to tissues supplied by those vessels (159). In fact, Stubblefield explains radiation injury as “myelo-radiculo-plexo-neuro-myopathy” due to the implicit and essential interactions between the musculoskeletal and nervous systems that allow for normal functioning of all involved tissues (163).

Radiation-induced fibrosis (RIF) is an irreversible progressive fibrotic tissue sclerosis that occurs months to years after RT ends and is often cited as a potential contributor to shoulder pain and

dysfunction as well as to decreased quality of life (33,38,42,58,75,150,163–165). Radiation induced fibrosis consists of an increase in collagen and extracellular matrix concentrations secondary to myofibroblast proliferation, with concurrent decrease in remodeling enzymes resulting in increased and progressive tissue fibrosis, decreased tissue compliance, and a reduction in local vascularity (38,125,150,159,163). Due to these adverse tissue effects, RIF is known to cause functional and cosmetic impairments and adversely affect quality of life (38,163).

Radiation damage to blood vessels can also affect tissue health and healing properties secondary to RT-induced vascular thickening, lipid accumulation, inflammation, and thrombosis secondary to continued oxidative damage (151). Radiation-induced nerve damage can include demyelination and fibrosis, and has the potential to affect muscle recruitment and activation (166). RIF may also predispose tissues to physical trauma (38) which must be taken into account as the patient returns to physical activities or exercise programs that can challenge potentially impaired tissue repair systems. Therefore, active and passive physiological characteristics of the affected tissues as well as their nerve activation and ability to heal, have the potential to affect shoulder function.

Liu et al used ultrasound to examine skin tissue toxicity secondary to breast-cancer radiation an average of 22 months post RT (125). The authors discovered a 27% increase in skin thickness and a 94.6% increase in midband fit, a reflection of increased collagen content, compared to the untreated breast following RT. Radiation dose was 50.0-54.0 Gy delivered to the entire breast followed by an electron boost of 10.0-16.0Gy to the tumor bed. Conversely, Wong et al found a 9% decrease in skin thickness compared to the non-irradiated side following a dose of 46-50Gy an average of 27 months post-RT (167). In that study, only one measurement was taken at each site as compared to other studies that found an increase in skin thickness where methodology involved multiple measurements (125,126). Another study by Lin et al demonstrated an increased breast skin thickness ratio (breast skin thickness of the affected/treated side versus the unaffected/untreated side), indicative of fibrosis and correlated with RTOG grading criteria of RT-induced skin toxicity, in women following ALND (not SLNB) following radiation(168).

Radiation-induced damage to healthy tissues is therefore complex and ranges from cosmetic (fibrotic, atrophied breast tissue) to pathological (increase in arm and breast lymphedema risk and increased risk of shoulder dysfunction, cardiac pathologies) (35,124,151,157,169). It can also increase the risk of breast reconstruction complications three-fold (170,171). The late effects of

radiation therefore have the potential to impact local tissue health and indirectly and adversely affect function of nearby joints such as the shoulder that depend on these tissues to function normally.

Radiation and Combined Therapies

Side effects of RT may be more common or severe when therapies are combined, as when chemotherapy is part of the treatment regimen (159). Radiation recall, an exaggerated radiation response that can involve increased erythema, fibrosis, or skin erosion, may be more pronounced with tamoxifen and Herceptin, 2 common medications used to treat certain types of breast cancer, or when chemotherapy and radiation are both used (150,172,173). However, the impact of hormone blocking medications plus concurrent RT on overall survival and tissue toxicities remains unclear, with some studies finding increased breast, lung, or cardiac fibrosis in those that have concurrent hormonal therapy + RT and other studies finding no difference in overall survival and fibrosis as compared to sequential hormonal therapy + RT, suggesting a need for a better understanding of this complicated relationship (174).

The relationships among estrogen, aging, and muscle health are complex, especially when taking into consideration forced estrogen deficiency by endocrine therapies in those with estrogen-positive breast cancers. Estrogen levels normally decline with age and menopause, which can negatively impact skeletal muscle function including decreased muscle mass, strength, and recovery following injury (175,176). Part of its role may be secondary to the protective effect of estrogen against oxidative stress (176,177), which as described previously, is an unavoidable side effect of RT. Estrogen also plays a significant role in satellite cell health; decreased estrogen levels in post-menopausal women can decrease skeletal muscle stem cell number which can then adversely affect muscle strength and recovery. In fact, in one study, satellite cell number was decreased by 30%-50% when estradiol was not present (175,178). As both estrogen depletion and RT have the potential to negatively affect satellite cell number, our study specifically included analyses of skeletal muscle morphological characteristics in those who had estrogen-blocking therapy.

Radiation Effect on Skeletal Muscle

Background and Dose Volume Histogram (DVH) Analysis

Radiation affects not just cancer cells, but also surrounding healthy tissues (including nerve, muscle, skin, and lymphatics) within the radiation field (25,52,150,159). The effect of radiation on

skeletal muscle (or any tissue) can be expressed by a combination of total dose, fraction size, and a ratio of irreparable damage (alpha) and repairable damage (beta) that represents specific tissue radiosensitivity (152). Precise CT-planning is performed for each patient, with the goal of targeting the cancerous area as well as other breast tissue that may harbor otherwise undetectable microscopic cancer cells while minimizing exposure to OARs. Unfortunately, healthy tissues such as skeletal muscles and their innervating nerves are also located within the radiation field (52,179).

Dose volume histogram (DVH) analysis is a method typically used to determine the predicted absorbed radiation dose for RT planning. This method, commonly integrated into radiation software, calculates the predicted absorbed radiation dose of tissues within the planned target volume. The data is presented as a plot of radiation dose (x-axis) against percentage of volume of tissue of interest (y-axis) for a specific RT treatment plan (180).

In 2017, Lipps et al was the first to individually measure and compare the effect of 5 different radiation treatment plans on the predicted absorbed radiation dose of 9 different muscles within the radiation field (52). They used pre-treatment CT scanning in 11 women undergoing treatment for breast cancer to individually contour and determine estimated radiation dose delivered to the infraspinatus, latissimus dorsi, PMaj, PMin, subscapularis, supraspinatus, teres major, teres minor, and trapezius muscles.

They demonstrated that whole breast irradiation caused the PMaj, PMin to absorb large doses (48Gy, near the prescription dose of 50Gy) while the addition of regional lymph node irradiation (supraclavicular and axillary nodes) significantly increased the dose delivered to the latissimus dorsi and the teres major muscles ($p < 0.001$). There was a main effect of radiation treatment plan on all muscles measured, signifying that the effect of radiation on each muscle (including the pectorals, latissimus dorsi, rotator cuff, and trapezius) may depend on the radiation plan utilized ($p < 0.001$). This finding is in agreement with existing literature, which shows that radiation treatment plan affects dose delivered to traditional OARs (153). Lipps et al stressed that the relationship between predicted absorbed dose and muscle function and/or force was not evaluated as part of this study but should be included in future research.

The relationship between predicted absorbed radiation dose and skeletal muscle morphology is not well understood. Radiation sequelae can include atrophy, weakness, loss of elasticity, fibrosis,

shortening, and contracture (32,33,40,51,181,182) as well as neuropathic damage including myelin changes, axonal degeneration, scar formation, and fibrosis (183). Human skeletal muscle cells are post-mitotic, suggesting that radiation may not directly affect adult skeletal muscle cell division directly(184,185). Instead, the effects of RT may be secondary to satellite cell DNA injury and strand breakage, causing satellite cell mitotic failure and cell death (185). This can negatively affect the skeletal muscle's ability to repair itself as the satellite cells are responsible for the repair of damaged muscle fibers (32,184,185). Furthermore, following acute radiation damage, the irradiated microenvironment is thought to adopt an aberrant healing response, possibly due to this satellite cell depletion and chronic inflammation (183,186). The effect of the initial RT-induced muscle damage amplified by impaired healing may affect not only muscle physiology but also muscle and adjacent joint and arm function.

Skeletal Muscle Inflammation, Thickness, Volume, and Stiffness

Wallner et al. performed muscle biopsies on the pectoralis muscles of 12 female breast cancer survivors at least 3 years after radiation therapy (50Gy) and Deep Inferior Epigastric Perforator flap reconstruction (162). This group used the rectus abdominis, which lays outside the radiation field, as a control. Radiated tissues had 67% normal myofibers compared to 92% in the control tissues. Additionally, compared to the rectus abdominis control tissues, radiated tissues had increased neutrophil infiltration, decreased growth and differentiation hormones, increased pro-inflammatory cytokines, and biomarkers indicative of muscle atrophy, scarring, and decreased myogenesis. This suggests chronic changes in radiated tissues that increase muscle catabolism and inflammation, which can negatively affect muscle structure and function.

The PMaj is one muscle that is within all breast radiation fields secondary to its location on the chest wall deep to the breast tissue itself. Seo et al found significant temporal volumetric changes in the PMaj muscle secondary to RT (41). They evaluated serial CT scans of 22 women undergoing RT for unilateral breast cancer at the following time points: before RT, immediately after RT, and 2 months, 6 months, 2 years, and 6 years after RT. Pectoralis major volume initially increased 2 months after RT as compared to pre-RT ($p < 0.001$), then decreased from 2 months to 6 months after RT ($p < 0.001$) and again from 6 months to 4 years after RT ($p < 0.001$). They also examined muscle volume surrounding the scapula and found no significant differences in muscle volume at any time point ($p > 0.165$ for pre-RT to post-RT, and $p > 0.999$ for all other time points). Seo et al suggested that RT-induced vasculitis, tissue injury, and / or denervation may have contributed to

their findings. They further emphasized the need for better understanding of possible causes of such volumetric changes to direct future treatment and/or rehabilitation.

Lipps et al used DVH analysis, US elastography, and Biodex measures of shoulder stiffness to demonstrate that the PMaj muscle was stiffer following postlumpectomy RT, especially when the surgery included ALND (versus the less involved SLNB) and when the radiation regimen included 3 or more lymph node fields (74). They examined both the clavicular and sternocostal regions of the PMaj and found significant correlations between V40 dose and muscle stiffness in both areas ($r = 0.54$, $p = 0.021$ for the sternocostal region and $r = 0.58$, $p = 0.012$ for the clavicular region). This study compared 3 groups of patients: those with lymph node involvement who had an ALND + RT to the breast and regional nodes, patients with a lumpectomy and SLNB + RT to the breast only, and healthy age-matched controls. One limitation was the significantly longer follow-up period for the first group (988 days vs 754 days, $p = 0.003$).

Wolfram et al followed 7 breast cancer survivors treated with lumpectomy, SLNB, and tangential field RT with boost before RT, then 30 days, 6 months, and 12 months after RT ended (42). Mean RT dose to the whole PMaj was calculated from RT simulation scans. All participants had hormonal therapy, and 1 had chemotherapy and hormone therapy. Using US elastography, they found that PMaj muscle stiffness was decreased 30days post-RT then increased at 6- and 12-months post-RT, suggesting tissue fibrosis. Using B-mode US, the sternocostal region of the PMaj also demonstrated decreased thickness, indicative of disuse atrophy. They defined muscle thickness as mean vertical distance between the deep and superficial aponeuroses at three distinct points of the PMaj. They also demonstrated that the sternocostal region of the PMaj received higher radiation doses than the clavicular PMaj.

Kim et al used a handheld myotonometer to detect increased tone, stiffness, and decreased elasticity of the affected PMaj compared to the unaffected PMaj in a group of 42 breast cancer patients evaluated before RT, immediately and 4 months after RT (187). The participants had either a lumpectomy or mastectomy, SLNB or ALND, and some had radiation to the breast alone and others to the breast and supraclavicular fossa. The location of the PMaj data collection was at the intersection of the vertical line from the clavicle midpoint and a horizontal line drawn from the axilla.

Other studies have also confirmed a relationship between RT and pectoralis tightness at 12 months post-treatment. Patients with pectoralis tightness (defined by limitations in forward flexion and horizontal abduction) at 3- or 6-month follow-ups had a higher incidence of rotator cuff disease at 12 months than those who did not exhibit those earlier traits (27.3% vs 4.3%, $p < 0.001$). In their discussion, the authors suggested possible etiologies of this tightness: subcutaneous fibrosis, hypertonicity secondary to post-surgical pain, radiation fibrosis, and thoracic flexion/scapular protraction, a posture that patients often adopt to subconsciously protect or hide their anterior chest surgeries (70).

Tissues of the anterior chest wall must stretch to allow for normal shoulder range of motion. Radiation fibrosis can thus affect shoulder motion via increased passive tension /resistance of the irradiated muscle. This could increase the difficulty of any movement that requires passive lengthening of that muscle. Conversely, it may necessitate increased contribution of antagonistic muscles to overcome that stiffness. Interestingly, Ryttov et al found that active but not passive range of motion was decreased in a group receiving mastectomy + RT as compared to a group receiving a mastectomy only , which may support the latter hypothesis (37).

A thorough understanding of RT effect on skeletal muscle health is thus essential to facilitate functional recovery after breast cancer treatment. The knowledge can not only help educate patients on how to safely return to daily activities but may also offer guidelines on how to safely progress patients into more strenuous upper body activities that may challenge an impaired muscle recovery system. It can also guide rehabilitative techniques by shifting imposed skeletal muscle stress to lower levels, thus avoiding overuse injuries that may happen at a lower threshold secondary to radiation damage.

Radiation Effects in Cancers Other Than Breast

Studies of radiation effects in different cancer populations provides other insight. He et al used electromyography (EMG) to evaluate the tensor veli palatine in patients with nasopharyngeal carcinoma with secretory otitis media after radiotherapy and found significant differences in the average duration and amplitude of the action potential, swallowing contraction duration and peak voltage, suggesting myogenic damage following RT (188). Van Leeuwen-Segarceanu et al concluded that there was myogenic damage within the radiation field and neurogenic damage outside the radiation field in patients who had received mantle field radiation treatment for

Hodgkin's lymphoma after needle EMG of the neck muscles in the radiation field revealed myogenic changes in 71% of their subjects (179). They also found significant sternocleidomastoid (SCM) muscle atrophy in 67% of their subjects but 'mostly normal' echo intensity of the SCM, suggesting microvascular fibrosis such as capillary damage that may be a primary contributor to the observed muscle atrophy. Additionally, 20% of their subjects had mixed neuropathic and myogenic damage of the neck muscles within the radiation field. They concluded that radiation-induced vascular injury may be partially responsible for the myogenic damage seen to irradiated muscles and suggested that specific muscle strengthening therapies may be able to reduce such damage by encouraging the growth of collateral vessels, with the goal of improving strength and function of in Hodgkin's lymphoma patients following radiation therapy.

The ability of radiation to affect tissues within the radiation field is a complex concept. Total radiation dose, radiation dose per fraction (dose given per day), chemotherapy, large-volume radiation plans, and postoperative complications (hematoma, infection) are a few relevant factors that can influence radiation effects (189,190). Patient-related factors that may affect radiation damage includes diabetes, hypertension, smoking, and increased age due to their influences on the vasculature, as well as genetics that are not yet fully understood (150,159).

In conclusion, there is sufficient evidence to support the idea that radiation can affect skeletal muscle on a cellular level which may lead to increased tissue stiffness and decreased strength. These changes can affect both active muscles, i.e. SA during scapular upward rotation, as well as passive muscles that need to stretch during motion, i.e. the PMin during scapular posterior tilt or the PMaj during horizontal abduction and overhead shoulder elevation via its attachments on the clavicle and humerus. Both active and passive modes of action may explain how radiation affects shoulder function in breast cancer survivors.

Radiation Therapy and Shoulder Function

The literature strongly supports the adverse effect of radiation on long term shoulder function including decreased upper extremity ROM (12,29,30,35,169,191), lymphedema (12,29,192–194), decreased shoulder strength (169,191), decreased general shoulder function (75,191,195), increased shoulder pain (8,17,196,197), muscle stiffness (74), adhesive capsulitis (198) altered shoulder kinematics (118), and decreased QOL (199).

Blomqvist et al measured isometric shoulder strength and range of motion in 75 women treated with mastectomy and axillary dissection for breast cancer, 30 of whom received postoperative RT. Measurements were taken on average 15 months following the completion of RT (169). They found that goniometric flexion and abduction ROM were significantly reduced in those receiving RT as compared to the group not receiving RT. They also found that all shoulder ROM measurements (flexion, extension, ER, IR, abduction, and adduction) were significantly less within the same patient when comparing the radiated to the non-radiated side. Flexion, extension, abduction, adduction, and internal/external rotation shoulder strength were measured by an isokinetic device (Orthotron II^{TM}). Those receiving RT had weaker internal rotation than those not receiving RT, but within-subject comparison of RT vs non-RT side demonstrated significantly decreased strength in all muscle groups except external rotation. Function was not different between groups but was measured only as 'yes' or 'no' and may have not been sensitive enough to capture true differences. As the authors compared surgery alone to surgery + RT, they concluded that RT is the primary cause for reduced shoulder ROM and strength in those receiving mastectomy and axillary dissection.

The Danish Breast Cancer Cooperative Group examined late treatment morbidity in 84 breast cancer patients who had participated in their earlier trials (29). Patients were, on average, 9 years post mastectomy with Level I and partial Level II lymph node dissection. During the earlier trials, the premenopausal/perimenopausal women had been randomized into 3 arms: chemotherapy + RT, chemotherapy alone, or chemotherapy + tamoxifen. The postmenopausal women were randomized into 3 groups also: tamoxifen + RT, tamoxifen alone, and tamoxifen plus chemotherapy. Radiation consisted of 50Gy given in 25 fractions, 5 fractions per week. Patient reported cases of shoulder disability were significantly higher in the RT-group (38%) versus no-RT group (5%), $p < 0.01$. Observed shoulder movement was also significantly impaired (qualitative assessment only), with 52% of the irradiated patients having impaired mobility versus 15% of the non-irradiated patients ($p < 0.01$). Additionally, patient-reported shoulder function was significantly worse in the RT group (16%) compared to the no-RT group (2%), $p = 0.02$. The only significant contributing factor to impaired shoulder movement as determined by logistic regression was radiation ($p=0.001$, odds ratio = 6.0).

The START trials examined patient-reported breast, arm, and shoulder symptoms and body image after different RT regimens for early-stage breast cancer including hypofractionation (196). They

found that approximately 20% of women experienced self-reported moderate or marked shoulder stiffness 5 years after RT ended, regardless of which RT regimen they received. Pain also affected one third of patients 5 years after RT ended. START A randomly assigned women to one of the following 3 treatment regimens: 50 Gy in 25 fractions of 2.0 Gy (control), 41.6 Gy in 13 fractions of 3.2 Gy, or 39 Gy in 13 fractions of 3.0 Gy. Start B randomly assigned women to the same control as well as 40 Gy in 15 fractions of 2.67 Gy over 3 weeks (hypofractionation). Further analysis of the shoulder and arm outcomes for axillary surgery and lymphatic radiotherapy did not affect analysis, so the statistics were run without adjustment for these potential covariates.

The questionnaire used in their study to judge arm dysfunction consisted of only 3 broad questions: arm or shoulder pain, difficulty moving the arm, and swelling in the arm or hand. These questions may not have been specific enough to capture true comprehensive shoulder dysfunction data. Changes in breast skin appearance did vary by treatment regimen, however, suggesting that RT-induced skin effects may be dose sensitive, with the lowest total dose having the least change in skin appearance. Overall, the authors concluded that there was a high prevalence of chronic arm symptoms after surgery and RT, but also that treating to 39 Gy using a larger fraction size of 3 Gy per fraction did *not* result in an increase in arm or shoulder pain, stiffness, movement, or swelling, and suggested that hypofractionation should be considered an acceptable alternative to the standard regimen of 50Gy in applicable cases.

Hidding et al performed a systemic review in 2014 including 39 studies from January 2000 to October 2012 to better understand which breast cancer related treatment effects had the most significant impact on UED at least 3 months after Stage I-III breast cancer treatment (8). Regarding reduced shoulder ROM into abduction, flexion, and external rotation, they reported Level I evidence for mastectomy and axillary RT, and Level II evidence for ALND and RT to the chest wall. Regarding muscle strength, ALND/concurrent RT and chemotherapy were reported as Level I evidence and SLNB, chest wall RT, and chest wall + axillary RT as Level II evidence. Regarding pain, they reported Level I evidence for ALND and RT before chemotherapy, and Level II evidence for SLNB and RT. In summary, the authors concluded that patients who had ALND had the highest risk of reduced shoulder ROM, reduced muscle strength, and impaired ADLs. It is important to recognize that evidence also existed for increased risk of pain, reduced muscle strength/ROM/ADLs in the SLNB + RT group although it was not as strong as ALND + RT. Interestingly, women who had hormonal therapy or RT were at higher risk of post-treatment pain.

Johansen et al examined the effect of lumpectomy, axillary dissection, and either 48Gy or 50Gy RT on late morbidity including arm edema, pain, shoulder strength, and working ability in a cohort of 266 women with Stage I-IIIA breast cancer (200). They found that RT increased the risk of decreased shoulder movement (RR = 4.6 (1.5 - 13.8, p = 0.007)) in women on average 6.6 years after treatment, although only 7% of women demonstrated decreased shoulder ROM. Older age also predicted decreased shoulder movement (RR = 2.7, p = 0.002). Shoulder motion was not qualitatively measured but was graded subjectively for flexion and abduction on a scale from 0-3 (no impairment, mild, moderate, or severe impairment), which could have influenced the results. Of note, axillary radiation also increased the risk of arm lymphedema (RR = 4.5, 1.8 – 11.2, p = 0.001).

Levangie and Drouin performed a systemic review of the literature including papers published between 1980 and 2008 with the goal of determining the effects of ALND and RT on shoulder function late effects (excluding lymphedema) (33). Twelve out of 375 potential articles were utilized that met their criteria as they reported means, standard deviations, confidence intervals, and / or odds ratios. Overall, the authors concluded that more extensive (nodal) RT was associated with increased shoulder morbidity, with possible contributing factors of radiation induced PMaj and damage such as fibrosis.

Johansson et al conducted a prospective 2-year study of 61 women whose breast cancer treatment included ALND alone, ALND plus breast RT, or ALND + breast RT + axillary RT (58). At the two-year follow-up, all groups had significant reductions in shoulder internal rotation (63% of patients), abduction (43%), external rotation (30%), and flexion (27%). However, only the ALND + breast/axillary RT group had decreased abduction, flexion, external rotation, and internal rotation at 6 months, 1 year, and 2-year follow up timepoints compared to preoperative measurements ($p < 0.001$). The authors concluded that the impaired ROM was secondary to ‘vascular string’ (axillary web syndrome, and / or stiff tissues in the affected chest wall, particularly in the PMaj and axillary regions.)

In contrast, a few studies have found no association between RT and upper extremity function. Siquiera et al performed a cross sectional study including 233 women on average 5 years after surgery and/or chemotherapy for breast cancer (14). Upper limb dysfunction was found in 55.4%

of women as measured by the Disabilities of the Shoulder and Hand (DASH) patient reported outcome functional questionnaire. Other measures were scar adherence, lymphedema, and the presence / absence of winged scapula. After the regression model was adjusted for age and BMI, only paresthesia secondary to intercostobrachial nerve damage was significantly predictive of upper limb dysfunction (OR = 1.96, 95% CI = 1.01 – 3.60, p=0.03). RT was not predictive of upper extremity function both before (p = 0.50) and after (p = 0.44) adjustment for age and BMI.

Despite this finding, the authors pointed out that according to other researchers, RT is a risk factor for decreased shoulder ROM after breast cancer treatment, but they did not offer an explanation why their findings differed. One possibility is that radiation was recorded only as occurring or not occurring and was performed in 72% of the subjects. It is possible that the broad categorization of 'RT or no RT', rather than utilization of regional lymph node radiation or estimated absorbed dose to muscles affecting shoulder function, was responsible for the inability of the DASH to detect a relationship between RT and shoulder function. Additionally, as only 18% of subjects did not receive RT, power may have been insufficient to find significant differences. The DASH may also have lacked sensitivity to detect a difference if it did exist.

Radiation Dose and Shoulder Function

Johansen et al specifically examined the relationship between RT dose to the arm and shoulder and arm/shoulder morbidity in 183 women who received surgery (lumpectomy or mastectomy followed by ALND) and RT for breast cancer (9). All participants in this study had extensive regional lymph node RT in addition to the breast or chest wall. Pre-treatment CT planning was analyzed using DVH analysis to determine dose delivered to the chest wall or breast tissue, as well as to the shoulder joint and immediate surrounding structures.

The breast and/or chest wall received 50 Gy and the regional lymph nodes received 46-50 Gy, with an additional 10 or 16 Gy boost to the tumor bed for women who underwent lumpectomy. The shoulder anatomy was delineated and defined by the outer contour of the humerus, coracoid process, and the acromion, with 0.5 cm margin added to include surrounding soft structures and the acromioclavicular joint. Arm morbidity was determined using Kwan's Arm Problem Scale, a patient-reported outcome. Shoulder flexion and abduction was measured using a goniometer and a side-to-side difference of more than 25° was considered to signify decreased mobility.

Less than 20% of the shoulder volume received >25Gy, with larger shoulder volumes receiving between 5Gy – 20Gy. They found that as the amount of shoulder volume that received 15Gy increased, so did the Kwan's Arm Problem Scale scores suggesting increasing dysfunction with increasing shoulder volume irradiated. Furthermore, shoulder volumes receiving 15Gy were correlated with arm pain, arm stiffness, swollen arm, use of arm, numbness, and shoulder abduction. However, when surgery was used as a covariate, only arm swelling and arm pain were significantly associated with 15Gy dose. The authors concluded that 91% had some degree of arm/shoulder morbidity following treatment and emphasized that radiation dose may affect shoulder function. It is important to note that some of the participants had extensive RT to the entire axilla, and others had RT only to the axillary apex due to change in RT methodology during the study.

Marazzi et al studied the effects of RT on shoulder function by looking at shoulder symptoms in women with breast cancer treated with surgery and adjuvant RT to the breast and regional lymph nodes at least 6 months after the completion of RT (195). They found that a mean dose to the shoulder higher than 7 Gy was significantly related to decreased DASH scores ($p < 0.001$). Like Johansen et al, they contoured the scapula-humeral articulation including the humeral head, glenoid cavity, and acromion-clavicle joint and ligaments to represent shoulder joint radiation exposure volume (9).

Bazan et al recently published a study in which they compared the effect of 2 radiation treatment techniques (intensity modulated radiation therapy (IMRT) vs. 3D conventional radiation therapy (3DCRT) on back and shoulder tissue radiation dose and related this to shoulder function as defined by the quickDASH (25). They defined the shoulder as an 'organ at risk (OAR)' secondary to its location within the radiation field, a term typically reserved for the heart, lungs and other vital organs (143). Their defined shoulder OAR included all muscles, soft tissues, bones, and vasculature from 2cm superior to the unilateral supraclavicular planning target volume to the inferior supraclavicular planning target volume slice of the pre-treatment planning radiation therapy CT scan. They then compared the shoulder OAR radiation dose to long-term (>6mo) shoulder function as measured by the quickDASH. They grouped all tissues, including the PMaj, PMin and SA among them and did not analyze the dose of these muscles separately. The authors concluded that, in patients receiving regional lymph node irradiation, IMRT resulted in significantly less shoulder volume exposed to 20–50 Gy as compared to those receiving 3D-CRT. Furthermore, they

concluded that those undergoing the IMRT program had lower quickDASH scores than patients undergoing 3DCRT, suggesting less shoulder dysfunction in the IMRT group.

As mentioned above, Lipps et al was the first to compare the effect of 5 radiation treatment plans on predicted absorbed radiation dose to 9 specific muscles within the radiation field, expanding on the consideration of muscles as OARs (52) They found that the absorbed dose of radiation by muscle within the planned target volume (PTV) varies by radiation therapy treatment regimen. As treatment volume such as the addition of regional lymph node irradiation increased, so did mean radiation dose to shoulder muscles, including 48 Gy dose which was close to the prescribed dose of 50 Gy. This group suggested that more research was needed to determine the association between this absorbed dose to muscles and shoulder function.

Radiation Therapy and Shoulder Function: Proposed Kinematic Rationale

Despite the understanding that adjuvant RT has the potential to affect healthy tissues within the planned target volume, and that RT has been shown to adversely affect shoulder function in many studies, we do not yet fully understand the underlying mechanisms. We must examine not only kinematics, or how the shoulder joint moves, but also the active and passive tissues that contribute to those motions, Borstad and Szucs et al. suggested that scapular motion is affected by postsurgical soft tissue restrictions (102). Ebaugh et al also proposed that soft tissue restrictions inclusive of capsuloligamentous and passive muscle tension can affect scapula upward rotation (93).

For example, the PMaj has its origin on the clavicle, sternum, superior 6 costal cartilages, and external oblique muscle and its insertion on the humerus, thus affecting both humeral and scapular movement, the latter via a coupling mechanism (47,97,201). As mentioned above, previous research has shown that RT increases stiffness of the PMaj as measured by shear-wave elastography (42,74) which has been implicated in the development of rotator cuff disease (3,70). Others have shown decreased PMaj length and/or increased tightness following surgery and RT (70). A short or stiff PMaj may inhibit the clavicle's ability to elevate, retract, and posteriorly rotate at the sternoclavicular joint as it would need to elongate throughout these motions. Decreased SC retraction may lessen ST external rotation and thus relatively increase ST internal rotation. Recently, using single-plane fluoroscopy and 2D/3D shape matching with finite helical displacement analysis, Lawrence et al. recently demonstrated that AC upwards rotation and SC

posterior rotation are the 'predominant' motions of ST upward rotation (97), suggesting that RT-induced changes have the potential to inhibit ST upward rotation. A tight PMaj also has the potential to limit humeral external rotation secondary to its insertion on the humerus; adequate humeral external rotation is needed to achieve normal shoulder elevation.

The PMin is also located within the standard radiation field and thus subject to radiation-induced tissue changes. Additionally, level I-III axillary lymph nodes surround the PMin (202) and are not only affected by SLNB (and more so ALND) but also by breast RT and more substantially, by regional RT (52). Its origin is on the 3-5th ribs and fascia over corresponding intercostal muscles, and its insertion lies on the superior edge of the medial border of the coracoid process (201). Radiation of the PMin may increase its inherent stiffness or fibrosis. Due to its anatomic attachments on the coracoid process, a stiff or short PMin can theoretically increase passive tension and limit AC joint motion, including posterior tilt, upward rotation, and internal rotation. Lawrence et al demonstrated that AC upward rotation was one of the predominant motions of ST upward rotation, demonstrating one potential mechanism by which an irradiated PMin can affect shoulder kinematics (97). Additionally, Borstad and Ludewig demonstrated that a short PMin, which they stated may be due to increased connective tissue among other etiologies, can result in decreased scapular posterior tilt and increased scapular internal rotation during arm elevation in the sagittal, coronal, and (scaption) planes in healthy individuals (203).

Shamley found that both the PMaj ($t=2.177$, $p=0.034$) and PMin ($t=2.289$, $p=0.026$) were smaller on the affected versus the unaffected side in women with breast cancer by utilizing MRI to measure cross sectional area bilaterally at T2, T4, and T6 levels (112). However, the breast cancer surgery and radiation treatments in this study varied widely, and time since treatment also varied (6 months to 6 years) which could have influenced the results. Further specificity of treatment is needed to better understand the effects of each breast cancer treatment type.

Part of the SA also lies within the traditional chest wall radiation field, but to the best of our knowledge, the amount of radiation that it receives during RT has not been quantified. It originates at the outer surfaces and superior borders of the upper 8 or 9 ribs and inserts onto the costal surface of the scapular medial border (201). The SA is essential for normal shoulder function as it works in conjunction with the lower trapezius to upwardly rotate the scapula during arm elevation (48,104). It also contributes to ST posterior tilt which is necessary for normal shoulder function

(96). In healthy individuals with shoulder impingement, the SA has decreased activity during arm elevation compared to a healthy cohort without shoulder impingement (48).

The SA is innervated by the long thoracic nerve, which originates at C5-C8. The long thoracic nerve is superficial, and as such may be subject to radiation damage although this has not been directly proven (204). Weakness of the SA can result in scapular winging, or prominence of the medial border of the scapula in addition to limited arm elevation and decreased scapular upward rotation. Scapular winging is not an uncommon finding following surgery for breast cancer (118,119).

Thus, fibrosis of the SA and altered nerve conduction both have the potential to decrease scapular upward rotation and negatively affect arm elevation. Prieto-Gomez et al demonstrated that women with persistent pain after breast cancer treatment demonstrate altered shoulder neuromuscular activity (204). They utilized surface electromyography to demonstrate decreased activity in the SA muscle in all tested conditions and attributed this finding to long thoracic nerve damage during surgery, with further negative effects due to radiation therapy and chemotherapy, fibrosis and scarring, and myofascial pain syndrome.

It would be beneficial from both diagnostic and treatment standpoints to better understand the relationship between radiation dose, tissue health, and upper extremity function. It is known that extremely high doses of regional lymph node radiation resulting in brachial plexus dose of 57Gy resulted in arm paralysis in 92% of breast cancer survivors (194). However, the amount of radiation that causes adverse or significant muscle effects is not clear but has been suggested to be much lower, at only 15Gy (9). This is substantially lower than standard radiation doses of 42.5Gy – 50Gy.

Bazan et al recently hypothesized that 1) higher doses such as 40Gy – 50Gy may be more likely to cause shoulder/arm morbidity, but also that 2) large volumes of tissues (such as muscles being studied in this proposal) exposed to lower doses of 5Gy – 10Gy may also have a significant detrimental effect on shoulder/arm morbidity (25). Therefore, it is important to study and understand the relationship of not just the highest dose delivered to each muscle of interest, but also carefully consider the proportion, or volume, of the muscles at risk exposed to lower doses. This can be achieved with dose volume histogram (DVH) analysis embedded in radiation oncology software, which is explained later in this paper.

In summary, the complex and multifactorial relationship between adjuvant breast cancer RT and shoulder function is not thoroughly understood. Adjuvant RT has the potential to affect shoulder motion and function stemming from its tissue-level effects on the PMaj, PMin, and SA as well as their direct and indirect effects on SC, AC, and ST kinematics. As shoulder function directly affects the ability of patients to comfortably perform ADLs that may range from carrying groceries to donning and doffing clothing overhead to carrying children or grandchildren, a better understanding of these concepts is necessary to maximize function and quality of life in breast cancer survivors.

Patient-Reported Outcome Measurement of Shoulder Function: the Penn Shoulder Score

The Penn Shoulder Score, or PSS, is a frequently utilized patient-reported functional score in the breast cancer population (see Appendix A) (53,63,103,191,205). The PSS is a 100-point patient reported outcome measure consisting of 25 questions and 3 subscales: function, pain, and satisfaction. Lower scores represent decreased function within each subscale. The highest score of 100 reflects high function, low pain, and high satisfaction with shoulder function. Reliability is high in a population of patients with shoulder disorders with test-retest $ICC_{2,1} = 0.94$ and minimal detectable change of 12.1 points based on a 90% confidence interval (206). The Oncology Section Task Force on Breast Cancer Outcomes highly recommended the PSS for the breast cancer population, in addition to 3 other patient-reported outcomes including the DASH and Shoulder Pain and Disability Index (SPADI) questionnaires (53).

Harrington et al examined shoulder function using both the DASH and the PSS as well as range of motion and strength in a population of breast cancer survivors without further delineation of surgery/radiation/reconstruction treatment. Both the PSS and DASH were able to show significant differences in function between the breast cancer survivor group and healthy controls (191).

The PSS was specifically chosen due to its specificity related to affected / injured shoulder function as opposed to the DASH and SPADI which include more vague questions that can apply to both shoulders, including the unaffected / uninjured side. For example, the DASH prompts for rating ‘push open a heavy door’ and ‘carrying a shopping bag’ while the PSS specifies ‘open a door with the affected side’ and ‘carry a bag of groceries with the affected arm’. The SPADI was also considered, but also does not differentiate based on laterality which was an essential component for this study.

CHAPTER 3: METHODS

Study Design

This was a single-center, non-therapeutic, cross-sectional study performed at the University of Minnesota between July 2022 and September 2023. The study was approved by the University of Minnesota Internal Review Board and the M Health Fairview Research Board Committee. All participants provided written informed consent prior to data collection.

The study was conducted in two sequential parts. First a pilot study was performed that included 5 healthy volunteers (Specific Aim 4). The purpose of this study was to determine feasibility and intra-rater reliability of musculoskeletal ultrasound to examine skeletal muscle morphology (EI and CSA) of the PMaj, PMin, and SA muscles. See Figure 5.

Second, a larger observational cross-sectional study was performed to better understand the relationships between radiation therapy, skeletal muscle morphology, shoulder kinematics, and patient-reported shoulder function in breast cancer survivors at least 1 year after the completion of adjuvant (post-surgical) radiation therapy (Specific Aims 1-3). See Figure 6.

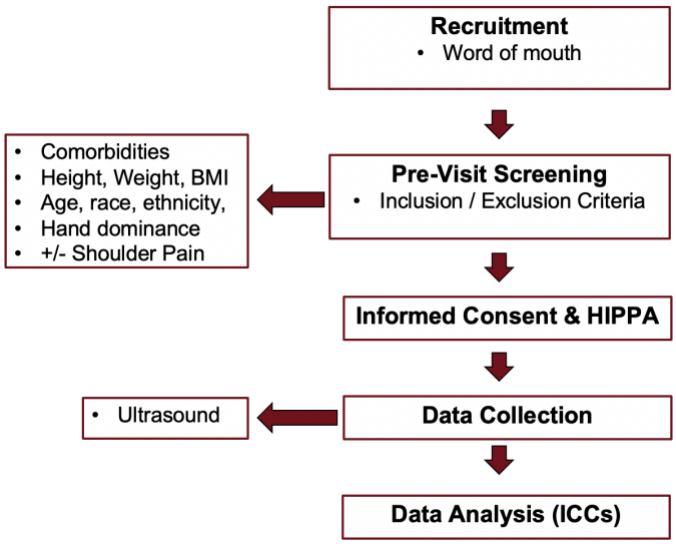

Figure 5. Flow diagram of ultrasound reliability study involving healthy volunteers (Aim 4).

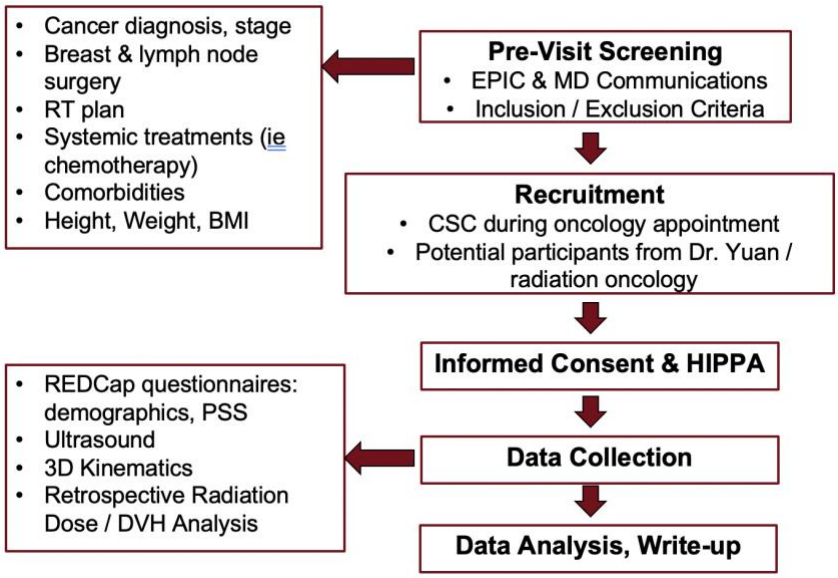

Figure 6. Flow diagram showing protocol for main study involving breast cancer survivors (Aims 1-3).

Recruitment

Five healthy volunteers without a history of breast cancer were recruited by word of mouth by RAB from July 2022 – August 2022 for the US reliability study. This was a pilot study designed to determine intra-rater reliability of US measurements that were then used for the main study involving breast cancer survivors. Inclusion criteria included age greater than 18 years and availability for testing at the University of Minnesota. Exclusion criteria included a history of shoulder problems, breast cancer, breast or chest wall injury or surgery, or neuromuscular or connective tissue disorders. A wide range of ages and body morphologies (young to old, low to high BMI) was intentionally selected by RAB to represent heterogenicity of the general population and to minimize selection bias that favored using young, lean participants which would likely lead to inflated ICCs due to ease of scanning. All participants provided voluntary written consent prior to any study related activities and answered a short demographic questionnaire on REDCap (see Appendixes B and H). These participants were provided with a $20.00 Target gift card plus paid parking for compensation of their time.

The breast cancer survivor cohort was recruited between November 2022 and April 2023 and included 30 patients treated within the M Health Fairview hospital system in Minneapolis, MN. See Appendix D for M Health Fairview required screening form. Potential participants were

recruited using one of two methods. The first method of recruitment was direct patient contact. Potential participants who had regularly scheduled oncology appointments at the Breast Cancer Clinic at M Health Fairview Clinic and Surgery Center in Minneapolis, MN were pre-screened in EPIC by RB to determine if they fit inclusion/exclusion criteria. Immediately following that oncology visit RAB met the potential participant, explained the research study, provided a research study brochure (see Appendix E), and answered any questions. Interested participants were consented by RAB at that time. See Appendix G. Those who were uncertain at the time of the meeting were instructed to contact RAB using the email provided in the brochure.

The second method of recruitment utilized a list of patients generated by Dr. Yuan (University of Minnesota Radiation Oncologist and co-PI), who received postoperative radiation therapy from Dr. Yuan or her team at M Health Fairview or the University of Minnesota for the treatment of breast cancer. Potential participants from that list who fit inclusion/exclusion criteria as determined by screening through EPIC were mailed a letter of introduction from Dr. Yuan and the rest of the study team; a study brochure was also included in the mailing. See Appendix F. The brochure described the research study and its rationale, along with basic inclusion criteria, data collection, time involved, and compensation. Interested participants either emailed RAB or called the Cancer Survivorship and Lymphology Laboratory. RAB would then email or speak to interested participants directly to explain the research study and answer all questions. Interested participants were scheduled for the in-person portion of the study, where written informed consent took place. All breast cancer survivors received a $40 gift card to Target plus validated parking following completion of the in-person testing.

<u>Inclusion and exclusion criteria for the breast cancer survivor study</u>

Inclusion criteria:

- Diagnosis of unilateral breast cancer and at least 1 year after completion of lumpectomy and SLNB followed by whole breast irradiation with or without regional lymph node irradiation
- Radiation pre-treatment planning CT available
- Age 18 years of age or older at the time of consent
- Provided voluntary written consent prior to any study related activities

Exclusion criteria:

- Known or suspected pregnancy
- Prior ipsilateral or contralateral breast surgery (for any reason including but not limited to cosmesis and cancer)
- Mastectomy (prior or related to most current diagnosis)
- Breast reconstruction (prior or related to most current diagnosis)
- History of contralateral or bilateral breast cancer
- Past medical history of ipsilateral or contralateral shoulder surgery or presurgical shoulder pain or injury that required medical intervention
- Prior radiation to the breast, chest wall or either upper extremity
- Axillary lymph node dissection
- Known adhesive allergy
- Non-completion of radiation therapy regimen
- History of neuromuscular or connective tissue disorders
- History of burn injury of the upper extremities, torso, or neck
- Breast cancer recurrence since completion of RT with prior or current therapies for recurrence

Medical Data Collection (breast cancer survivor cohort only)

The following demographic and medical history data were collected from EPIC, a HIPAA compliant electronic health records system used by M Health Fairview. All data attained in EPIC was securely stored in the University of Minnesota's secure, PHI-compliant data collection system, Box Secure Storage.

- Sex
- Age
- BMI
- Hand Dominance
- Race / Ethnicity
- Smoking
- Diabetes
- Menopausal Status
- Affected Side
- Cancer Stage
- CTCAE skin reaction*
- Breast Surgery
- Lymph Node Surgery
- Number of Lymph Nodes Removed
- Number of Positive Lymph Nodes
- Chemotherapy
- Endocrine Therapy
- Radiation Treatment Plan
- Radiation Dermatitis Grade
- Occupation

- AWS

* Common Terminology Criteria for Adverse Events (CTCAE) skin reaction grade at the end of radiation (NIH NCI CTCAE2017). CTCAE skin reaction grade refers to a grade given to skin reaction resulting from radiation and was recorded in EPIC by Dr. Yuan, the treating radiation oncologist. Skin reaction grades range from 1-5, with 1 signifying mild symptoms not requiring intervention, 2 (minimal, local, or noninvasive symptoms requiring intervention), 3 (severe or medically significant but not immediately life-threatening that requires hospitalization and interferes with self-care and ADLS), 4 (life-threatening with urgent intervention needed) and 5 signifying patient death.

Study sequence

Healthy participants who participated in the US reliability study also answered a basic demographic REDCap questionnaire. See Appendix B and Figure 7. Breast cancer survivors participated in US, 3D kinematics, REDCap questionnaires, and gave permission for RAB to access and analyze pre-existing radiation CT scans that were a part of their prior breast cancer treatment. See Figure 8.

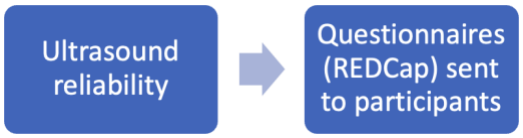

Figure 7. Procedure sequence for ultrasound reliability study.

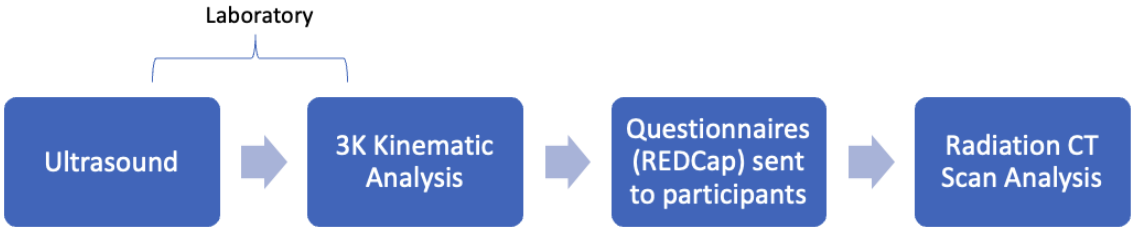

Figure 8. Procedure sequence for breast cancer survivor study.

Instrumentation and Procedures

Ultrasound Instrumentation

A portable B-mode US imaging device (Sonosite PX, FUJIFILM Sonosite Inc, Bothell, WA) with a L15-4 transducer was utilized to obtain images of the PMaj, PMin, and SA muscles bilaterally in both breast cancer survivors (main study) and healthy participants (pilot study). These muscles were specifically chosen due to 1) their location within the radiation field and 2) their anatomical

origins and insertions onto the scapula, humerus, and ribs that have the potential to influence shoulder kinematics (48,93,104,105,108). RAB performed all ultrasound imaging.

The L15-4 transducer has a linear head with 15Hz-4Hz capabilities and a scanning depth of 1cm - 6cm which allows scanning in the preferred musculoskeletal range of 5-7MHZ as cited previously (129). The Sonosite PX preprogrammed Musculoskeletal Program Mode was selected for all data collections. Each muscle had a preselected region of interest (ROI) that was easily reproducible (exact region of interest locations are further explained below). The ROI for each muscle was chosen during US training prior to the first study to maximize precision and to minimize error to allow side-to-side and between-participant comparisons. Ultrasound settings including gain, depth, and frequency were kept constant within each participant and were chosen prior to each participant's actual image acquisition based on image quality. Depth varied between participants due to body morphology / subcutaneous tissue volume but was held constant within each participant to minimize within-subject error and to allow within-subject comparisons.

US Data Collection

Ultrasound data collection was performed at the Cancer Survivorship and Lymphology Research Laboratory at the University of Minnesota. Both healthy participants and breast cancer survivors participated in the US portion of the study, the former to obtain intra-reliability data that was used to validate use of this technique in the breast cancer population as the methods were novel and adapted from other procedures as explained below.

Prior to image collection, all participants lay supine for at least 15 minutes to allow fluid shifts to occur (130). During that time, RAB marked the desired scan locations bilaterally using a skin marker, reviewed the US procedure and answered all participant questions. The order for examination was as follows for every participant: right PMaj and PMin, right SA, left PMaj and PMin, and left SA. All images were taken at the end of natural expiration to control rib and muscle movement (207). See Figures 10-12 for exact skin marking and ROI location methodologies for each muscle.

Scanning technique was consistent for all scans to maximize reliability. The coracoid process was chosen as the starting point for all scans, as the use of a bony landmark maximizes reliability and is commonly utilized in anesthesiology (208). The transducer head was positioned perpendicular

to the skin to minimize anisotropy (angle-generated artifact) and probe orientation was held constant for each muscle group (128). All US measurements were performed using gel as a coupling medium as sound waves do not travel through air and marginal pressure was applied with the transducer to minimize underlying tissue distortion (128).

Three independent images were taken of each muscle for the intra-rater reliability study, with the PMaj and PMin measured in a single image, resulting in 12 images per healthy participant and a total of 60 images used for the reliability study. The images were blinded for analysis by Dr. Linda Koehler and given to RAB for CSA and EI analysis. After CSA and EI analysis, RAB was unblinded and measurements were utilized for the reliability calculations in the healthy cohort. One to three images were taken in the breast cancer survivor cohort to ensure a viable image was captured. If more than 1 image was taken in the breast cancer cohort, the image with the best visualization of the tissues of interest was used for analysis.

Pectoralis Major and Minor Scanning

Participants lay supine with their arms extended comfortably at their arms at their sides with their forearms pronated / palms facing down as per Pareja-Blanco et al (209). The scanning technique was modified from the anesthesiology pectoralis minor / PECSI plane block as per Mounir-Soliman (208). The coracoid process was palpated, and a skin marking pen was used to indicate a point 1cm medial and just superior to the clavicle to avoid gel interference and marker obliteration while permitting easy visualization during scanning.

The US probe was initially positioned vertically with the superior edge on the clavicle and inclusive of the coracoid process. Color doppler was utilized to visualize the axillary artery and vein, thus ensuring that the rib inferior to this was the 2nd rib (208). The probe transducer was then rotated approximately 45 degrees so that its orientation was inferior-medial/superior-lateral in alignment with deltopectoral groove to allow for better rib visualization. The probe was moved inferiorly until the 2nd and 3rd ribs were completely within the field of view, and then slid medially to be in line with the previous mark made 1 cm to the coracoid process. The pectoral muscles' field of view was then defined by the skin superficially, lung pleura deep, and the 2nd and 3rd ribs cranially and caudally respectively, with the probe centered 1cm medial to the coracoid process (See Figure 9). The PMaj image was therefore taken in short axis view (perpendicular to the muscle fibers) and the

PMin was taken midway between short and long axis (parallel to the muscle fibers) to allow both pectoral muscles to be collected in a single image.

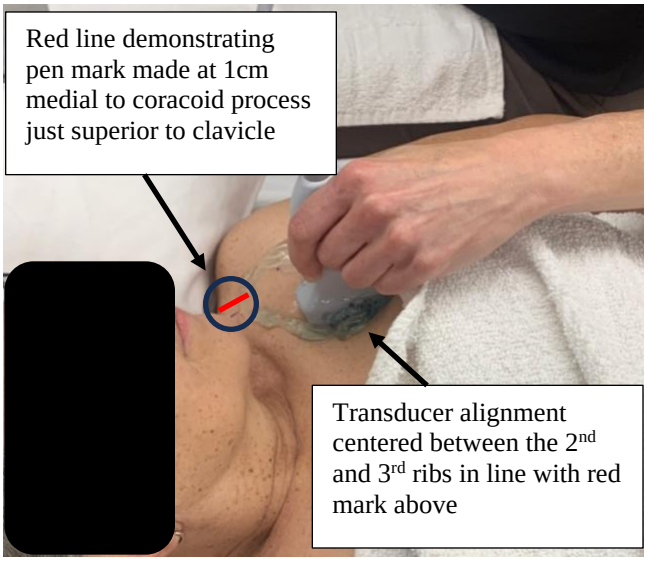

Figure 9. Ultrasound transducer head placement for pectoralis major and minor muscles image capture.

The image was labeled and 'frozen' on the Sonosite screen, then saved for download and analysis after completion of the entire scan. This procedure was repeated three to four times for the pectoralis muscle group to ensure satisfactory data capture and quality. See Figure 10.

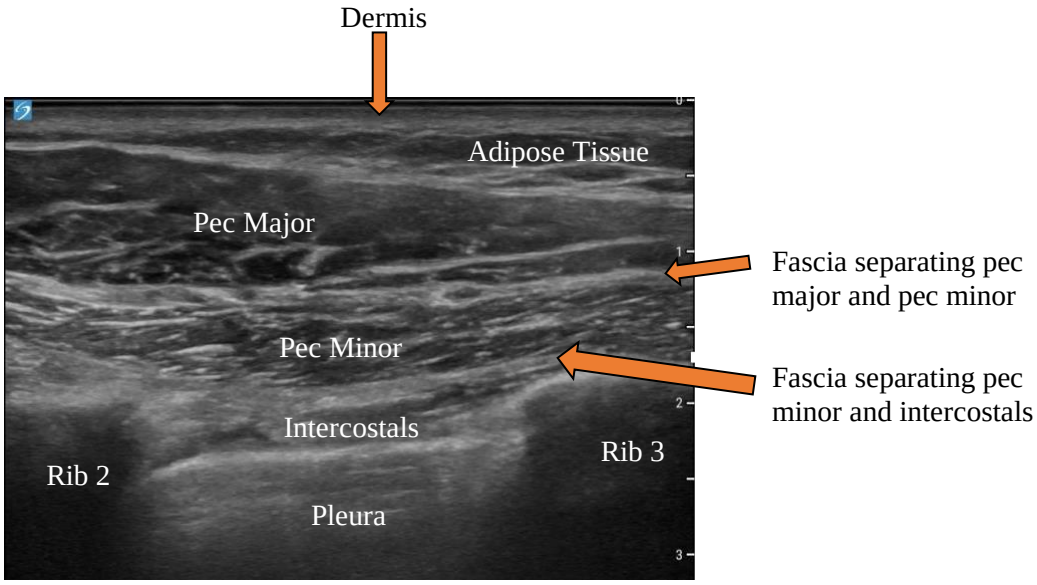

Figure 10. Sonosite image capture of the pectoralis major and pectoralis minor muscles with the participant lying supine, with the dermis, adipose tissue, intercostals, pleura, rib 2, rib 3, and intermuscular fascia labeled. The left-hand side of the image is more cranial, the right-hand side of the image is more caudal. The top of the image is ventral, and the bottom of the image is dorsal.

Serratus Anterior Scanning

After the PMaj group was examined on the right side, the participant continued to lay supine, and their shoulder was moved into 90 degrees abduction and 90 degrees external rotation with the hand dorsum resting on the pillow. A location 2 cm ventral to the midaxillary line was indicated with the skin marker just cranial to the axillary fold to prevent gel distortion and marker smear during scanning. We chose this site, 2cm ventral to the midaxillary line, to ensure that the SA was captured within the radiation field (44).

Scanning originated using the L19-5 transducer aligned vertically at the clavicle and coracoid process as previously described, confirming the location with the axillary artery and axillary vein. The transducer head was angled medially to allow for better rib visualization of the second rib and the ribs were counted as the transducer was moved inferiorly and laterally towards the midaxillary line as per Blanco et al as described for the anesthesiology serratus plane block (210). When the transducer was located over the 5^{th} and 6^{th} ribs, it was rotated parallel to the patient's thorax and moved ventrally to align with the point previously marked 2 cm anterior to the midaxillary line. The transducer was oriented perpendicular to the SA to capture the short axis image with 5th and 6th ribs in view and centered 2cm anterior to midaxillary line. The image was then 'frozen' on screen and saved for later analysis. See Figure 11.

The location between the 5^{th} and 6^{th} ribs was chosen based on preliminary data collection and revised from an anesthesiology SA nerve plane block to allow for visualization of the SA in its most superficial position, without overlay of the latissimus dorsi (210,211). During US training, it was discovered that we could not utilize the long axis view (parallel to the SA muscle fibers) as the transducer is flat and did not retain contact with the curvature of the ribcage at that location.

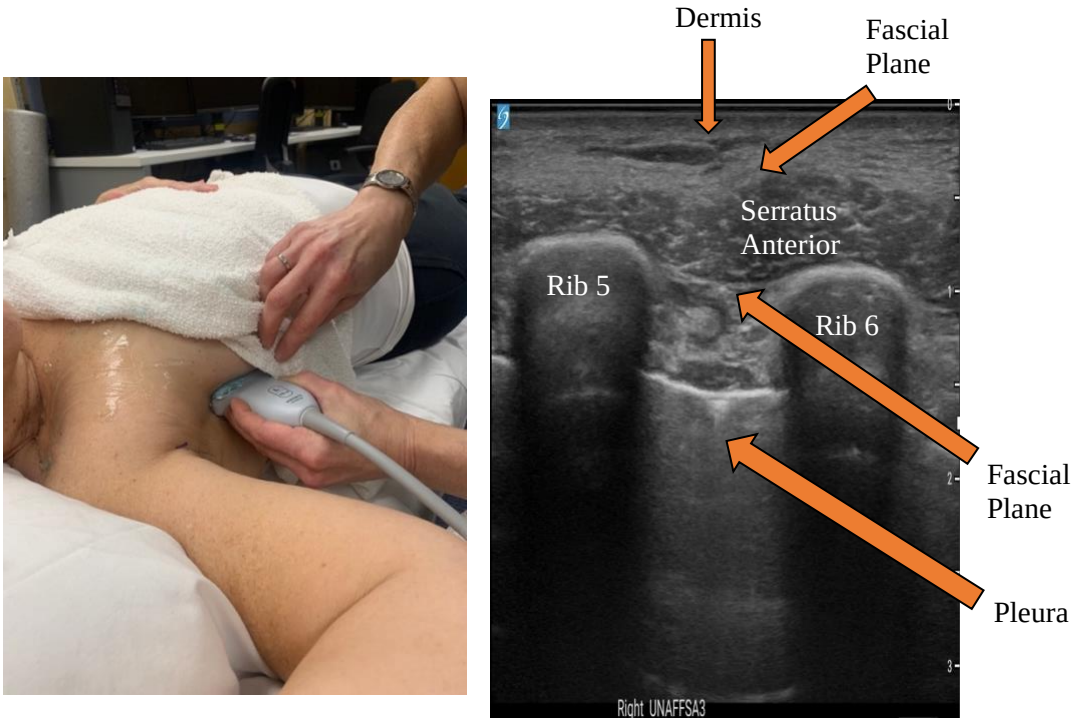

Figure 11. Serratus anterior ultrasound scan technique with the participant positioned supine with her arm abducted and externally rotated to 90 degrees, and the transducer aligned in short axis alignment to the serratus anterior between the 5th and 6th ribs at 2cm anterior to the midaxillary line.

Axillary Web Syndrome (AWS) Assessment

After the US assessment, participants continued to lay supine for examination by RAB to determine AWS status. AWS is a thin cordlike structure or structures that can be visualized or palpated subcutaneously in the axilla, upper extremity, and/or chest following lymph node removal during breast cancer surgery (212). RAB has extensive (20 years) of experience identifying AWS and has also assisted with and is a first author in AWS research with a nationally recognized expert (Dr. Linda Koehler, University of Minnesota) The arm was abducted fully with the elbow extended to allow for detection of the structure if present. Presence was confirmed by palpation with and / or without visualization of at least 1 cord in the axilla. AWS was marked as 'present' or 'absent' for both arms on a master data spreadsheet in Box.

3D Shoulder Kinematic Analysis Instrumentation

Breast cancer survivors participated in 3D shoulder kinematic analysis using the Flock of Birds® (Ascension Technologies Corporation, Shelbourne, VT) in the Cancer Survivorship Laboratory in the Children's Rehabilitation Building at the University of Minnesota. All testing was performed

by RAB with assistance from Dr. Paula Ludewig as needed. Flock of Birds® works by utilizing an electromagnetic field to induce current into sensors (2.54cm x 2.54cm x 20.3cm, 17g) made of 3 orthogonal copper coils that allows each sensor to have their own 3D coordinate systems. Flock of Birds® per Ascension technologies accuracy data is as follows: static accuracy (position 1.8mm, RMS orientation 0.5^0) and static resolution (position 0.5mm at 30.5cm, orientation 0.1^0 at 30.5cm) within a 76.2-cm range with an update rate of 144 measurements per second.

The Flock of Birds® transmitter was secured by plastic screws and Velcro onto a horizontal shelf extending from a plastic pole as metal may cause interference (85). The height of the transmitter on the pole was adjusted at every trial to ensure the transmitter was located at the central acromial height of each participant. Participants stood directly in front of the transmitter facing anteriorly to match the coordinate system of the person to that of the transmitter. See Figure 12. A pre-measured string with a 72-cm length was used to ensure that the participant's scapula and humerus of interest were within the 72-cm hemispheric field for all data collection.

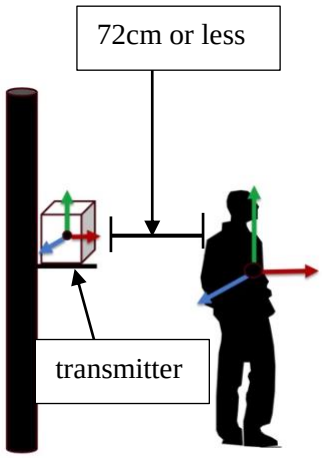

Figure 12. Orientation of participant to global (transmitter). Note both axes and coordinate systems directions are equivalent (red=x-axis positive anteriorly, green=y-axis positive vertically, blue=z-axis positive to the right).

Five Flock of Birds® sensors were placed on participants in sitting as per previous studies: (1) on a pointer, or stylus, with a known tip offset to allow for digitization of anatomical landmarks, (2) on the sternum just inferior to the sternal notch, oriented vertically, (3) on the superior surface of the medial half of clavicle, oriented on top of and parallel to the clavicle (4) on the flat surface of the posterior-lateral acromion oriented horizontally, and (5) on the distal humerus using a thermoplastic

cuff, oriented vertically. See Figures 13-15 (48,92,213). All sensors except that of the humerus were adhered to the skin with double sided tape as per Ludewig and Cook and kept in place using micropore tape to secure the sensor to the underlying skin (48). The humerus sensor was secured between two screws onto the thermoplastic cuff and attached to the arm with Velcro straps (Velcro USA Inc., Manchester, NH). The sternal sensor was secured with double sided foam tape to prevent slippage. See Figure13. The digitizing stylus with attached sensor was used to locate preselected landmarks per the International Society of Biomechanics (214).

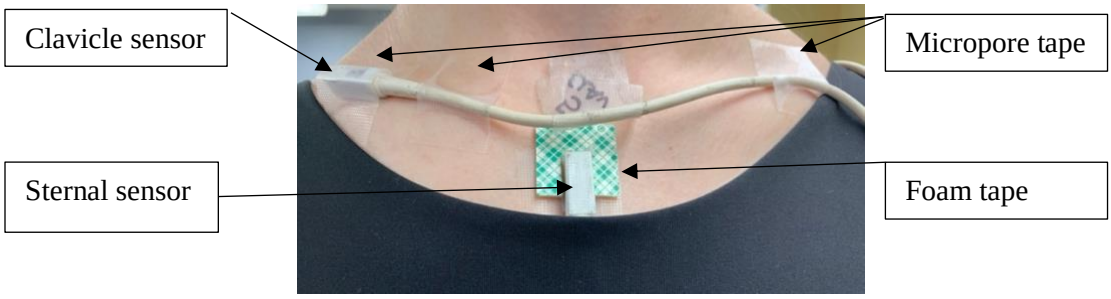

Figure 13. Flock of Birds clavicle and sternal sensor setup as demonstrated on the anterior superior chest wall. Clavicle sensor (to the left of the picture, located on right clavicle) held in place by double sided tape ventrally, and micropore tape superiorly and distally with a second piece placed on the left superior thorax to offset strain. Sternal sensor also held in place against the skin using double sided tape, with micropore tape distally and a second piece placed on the abdomen to offset strain. A piece of foam tape was cut and placed superior to the sternal sensor to prevent skin slip.

Micropore tape was also used for tension offset. An additional piece of micropore tape was placed a few inches proximal to the humeral cuff to secure the cord to the upper arm. Tape 'tunnels' were secured to the skin a few inches away from the posterolateral acromion (PLA) and clavicle sensors and used to guide the cords. A piece of micropore tape was also placed on the abdomen to offset any pulling on the sternal sensor.

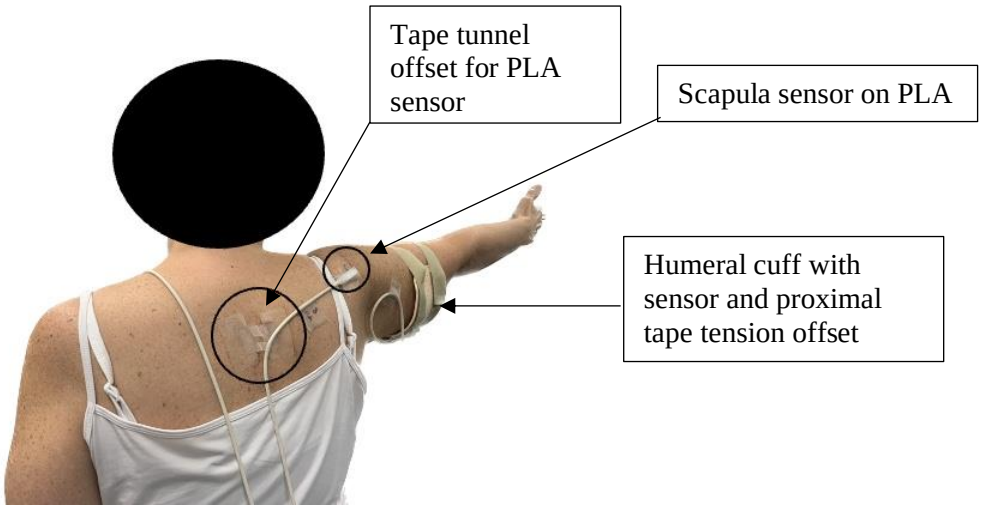

Figure 14. Posterior Flock of Birds setup demonstrating scapular (posterolateral acromion/PLA) sensor and humeral cuff with sensor. Tape offsets shown to assist with noise reduction.

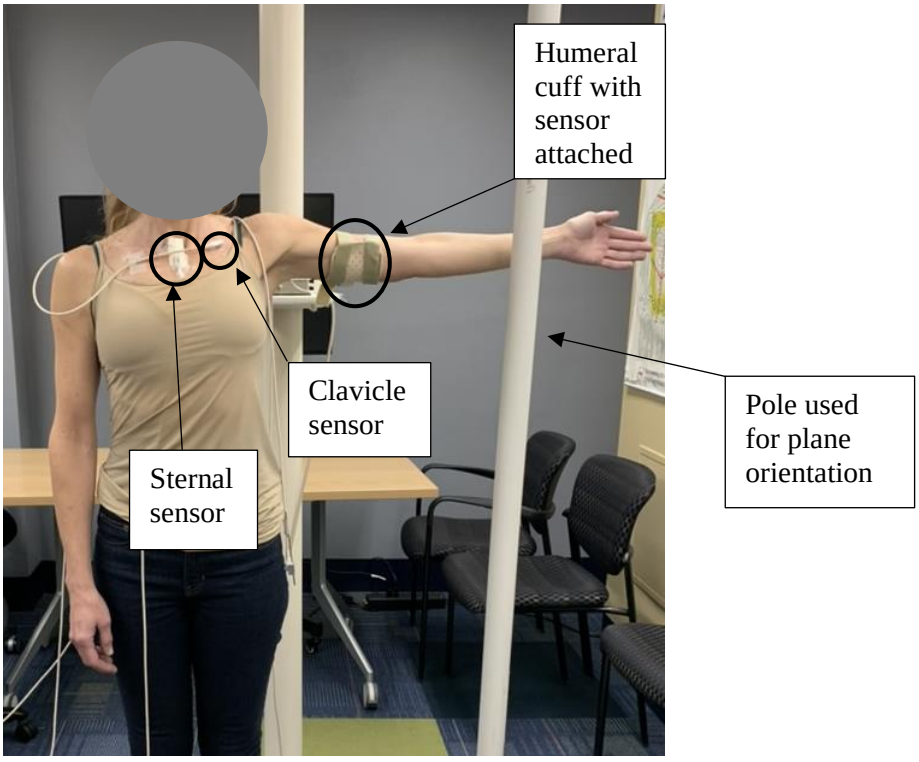

Figure 15. Anterior Flock of Birds setup demonstrating participant with sternal, clavicle and humeral sensors. Pole used to guide participant arm in frontal and abduction planes also shown.

Coordinate Systems

The global coordinate system was the Flock of Birds transmitter, with the x-axis positive anteriorly, the y-axis positive superiorly, and the z-axis positive to the right. The stylus with attached Flock of Birds sensor was used to digitize predetermined anatomical bony landmarks as per Wu et al (214). Digitization of these landmarks allowed orientation with respect to the FoB sensors (83). These landmarks were then used to build local coordinate systems for the thorax, clavicle, scapula, and humerus (48,83,214) as three non-collinear bony landmarks are needed to construct each local coordinate system.

The scapula coordinate system was built as per Wu et al utilizing the PLA, root of the scapular spine, and the scapula inferior angle(214). The scapula x-axis was positive anteriorly, the y-axis was positive superiorly, and the z-axis was positive to the right. See Figure 16.

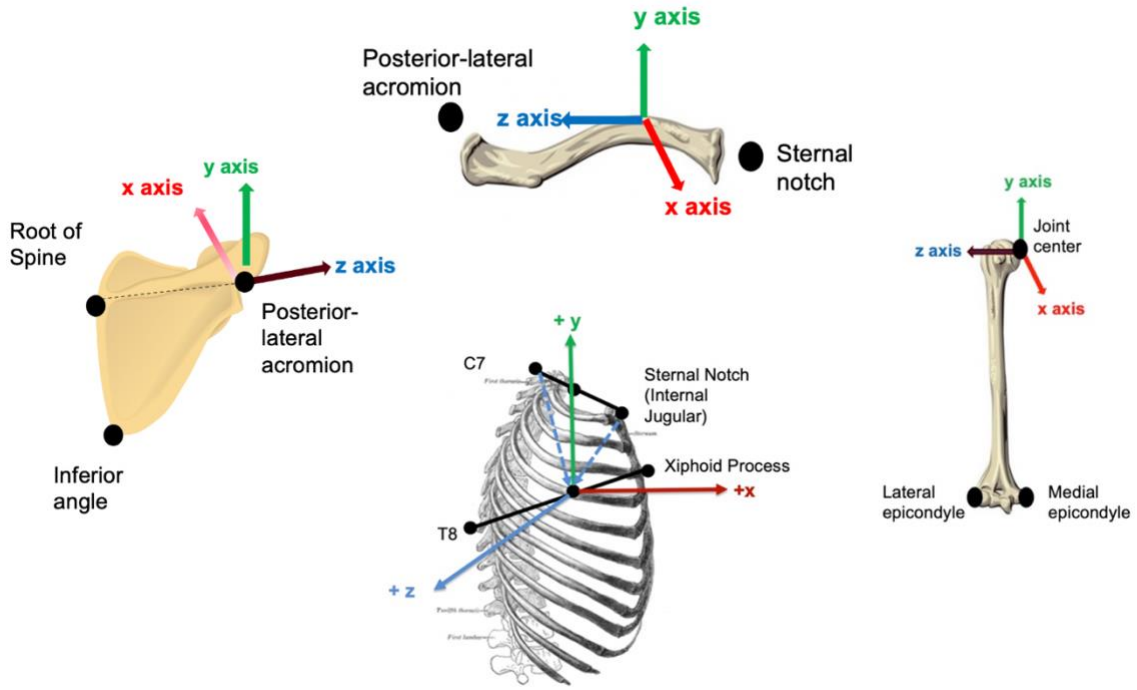

Figure 16. Scapula, clavicle, humerus, and thorax coordinate systems. The scapula x-axis was positive anteriorly, the y-axis was positive superiorly, and the z-axis was positive to the right.

The humerus coordinate system was built using the most caudal points of the medial and lateral epicondyles and the axis of rotation of the humeral head center as calculated by the least squares method (91). The humerus x-axis was positive anteriorly, the y-axis was positive superiorly along the shaft of the humerus, and the z-axis was positive to the right parallel to the axis between the medial and lateral epicondyles.

The thorax coordinate system was built from the suprasternal notch, spinous process of the 8th thoracic vertebra, spinous process of the 7th cervical vertebra, and xiphoid process. The thoracic x-axis was positive anteriorly, y-axis was positive superiorly, and z-axis was positive to the right.

The clavicle coordinate system was built using the most ventral portion of the sternoclavicular joint, the most dorsal portion of the acromioclavicular joint, and a third point superior and perpendicular to the clavicle between the SC and AC joints using a premade jig (5-1/2" Plastic Post & Pipe Multilevel by Empire Level, a division of Milwaukee Tool, Mukwonago, WI) as per the International Society of Biomechanics (214). See Figure 17. A point was digitized at the top corner of the jig cranial to the clavicle to create a third position that allowed building of the local clavicular coordinate system, with postprocessing corrections performed to adjust the clavicle vertical axis to the vertical axis of the thorax also per Wu et al(214).

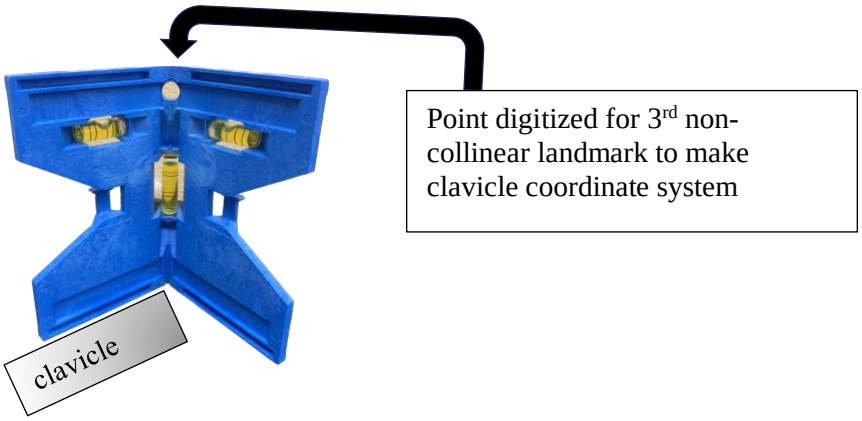

Figure 17. Clavicle jig used as an intermediate axis in building the clavicle coordinate system. In this photo it is positioned parallel to and on top of right clavicle.

3D Kinematics Data Collection

Both static and dynamic data collection were performed in standing. All data were collected at a 100 Hz sampling rate per sensor. Static data collection was performed for 5 seconds prior to dynamic movements and saved as a separate file. Participants were cued to stand in a relaxed position with their arms resting comfortably by their sides while resting data was captured.

Following static data collection, dynamic data collection was performed. First, three practice trials were completed in each plane prior to data capture. Participants were cued to run the tips of their fingers parallel to a planar surface aligned within the desired plane (flexion or abduction) while maintaining full elbow extension and humeral lateral rotation ("thumb up"). They were instructed not to touch the pole to avoid natural movement deviation. Participants were asked to elevate their arm as high as they were able, completing the movement concentrically and eccentrically over 3 seconds regulated by a metronome. Motion testing was performed three times with a 10 second rest between trials. The sequence (practice followed by data collection) was then repeated for abduction, with the planar surface moved to ensure alignment in the coronal plane.

Kinematic testing was performed on the right side first for all participants. After dynamic data collection was completed for the right side, the scapula PLA sensor, humeral cuff and sensor, and clavicle sensors were moved to the left side. The sternal sensor was not moved. The process was repeated for digitization of landmarks to make local coordinate systems for the left side, and the resting and dynamic movement performed, and data collection completed as above.

The Flock of Birds® was integrated with Motion Monitor Software (Innovative Sports Training Inc, Chicago IL) to capture, analyze, and visualize movements during 3D kinematic analysis. Data was collected on both the affected and unaffected sides in all breast cancer survivors and included humerothoracic (HT) arm elevation angles (the angle between the humerus and trunk), scapulothoracic (ST) rotations (scapula angles on the thorax into upward/downward rotation, internal/external rotation, and posterior/anterior tilt), and sternoclavicular (SC) rotations (rotation of the clavicle on the sternum into elevation, posterior rotation, and retraction).

Questionnaire Instrumentation and Data Collection

General demographics were collected for the US reliability study using REDCap, a secure web application used by the University of Minnesota. This included date of birth, gender, handedness, race, height, weight, and history of shoulder pain.

REDCap was used in the breast cancer survivor cohort for 2 questionnaires: the Penn Shoulder Score (PSS, see Appendix A) and a custom questionnaire designed for this study by RAB to better understand the participants' experience with and perception of shoulder problems (if present) and self-reported adjuvant radiation therapy treatment sequelae. (See Appendix C).

The custom questionnaire obtained self-report of the following information (see Appendix C for questionnaire):

- Race
- Handedness
- Skin irritation
- Onset of symptoms after radiation
- Medical treatment sought during or after radiation to address shoulder/chest wall symptoms
- Smoking
- Estrogen deprivation therapy
- Current or past problems in affected arm, shoulder, or chest wall
- Self-reported lymphedema history
- Menopausal status

CT Radiation Simulation Scan Instrumentation and Data Collection

Retrospective analysis of CT simulation scans for the breast cancer survivor study participants was approved by the University of Minnesota and M Health Fairview and performed by RAB. CT simulation scans are part of standard treatment planning methodology for all patients prior to radiation treatment. These scans allow individualized target beam design with maximization of dose delivered to the cancer while sparing OARs such as the heart and lung (143). These same CT simulation scans were analyzed to determine the estimated absorbed radiation dose of selected muscles at risk including the PMaj and PMin as initially performed by Lipps et al (52).The SA was also segmented due to its location on the chest wall, partial inclusion within the radiation field, and its vital contribution to normal shoulder movement. All CT scans were performed at M Health Fairview / University of Minnesota East Bank Radiation Oncology Department using a Philips "Big Bore" CT Simulation Scanner (Koninklijka Philips, N.V.)

The 3-dimensional conformal radiation therapy (3D-CRT) technique was used for all participants in this study. 3D-CRT is a computer-generated, physicist-modified image of target beam alignment that utilizes precise tumor and OAR locations. Prescription dose is delivered to the clinical target

volume, which typically includes the entire breast, as well as regional nodes when indicated. Planned target volume is the addition of a second margin to compensate for organ motion and positioning challenges (215). Axial CT images were taken every 3mm from the mandible to 5cm below the inframammary fold. Total prescription dose was patient dependent and consisted of breast only or a combination of breast and regional lymph nodes, with an additional tumor bed boost depending on tumor status and other factors as determined by the treating radiation oncologist.

Data Handling and Data Reduction

US Data Handling and Reduction

All US data were de-identified and transferred by USB to a computer for analysis. Skeletal muscle CSA and EI were measured using ImageJ software (version1.53k; Wayne Rasband and contributors, National Institutes of Health, Bethesda, Maryland) as described by Rosenberg et al (131).

In ImageJ, the image was first converted to an 8-bit image to allow for easier black and white processing. Then, a straight-line tool was used to scale each image from pixels to centimeters as this was the desired unit for cross sectional area analysis. This was done by drawing a line from 0-1cm in depth on the frozen image and assigning that length to 1cm, allowing conversion from pixels using that specific ratio of pixels:cm for every measurement in that specific picture as per Tanaka et al. (132). See Figure 18. This process was repeated for every image analyzed to ensure scaling was specific to each individual image.

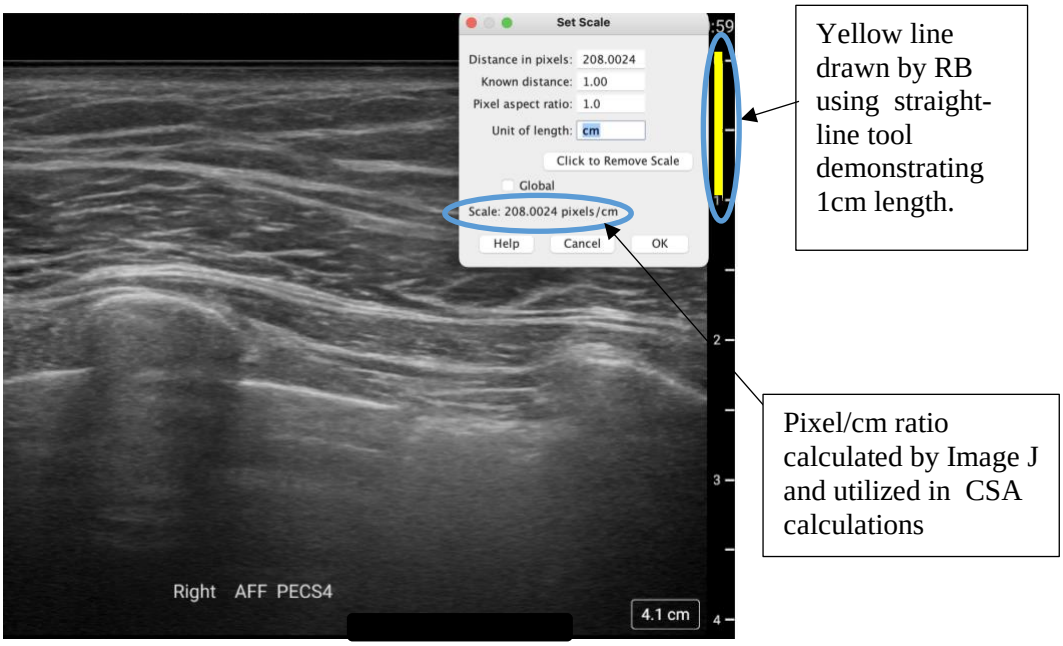

Figure 18. Screenshot of Image J straight-line scale tool used to convert ultrasound raw data pixels to centimeters.

Region of interest boundaries for all muscle groups were selected using the polygon function and used for both CSA and EI analyses (121,131). The ROI of the pectoral muscles was defined as follows: the superior and inferior boundaries were the outermost edges of the cortex of the inferior border of the second rib and the superior border of the third rib. Superficial and deep boundaries were delineated by and exclusive of the fascial borders of each muscle, clearly visible by the white appearance of the epimysium at the perimeter of the muscle. In a few cases where the fascial border was not easily recognized, the image brightness and contrast were temporarily adjusted until the borders were more easily visible.

CSA and EI were then determined within each ROI using the Analyze/Measure function and was expressed as 'Area' and 'Mean' on the Results tab respectively. Echogenicity was reflective of values from 0 (black) to 255 (white)(121). These techniques were adopted from Rosenberg et al (131). See Figure 19.

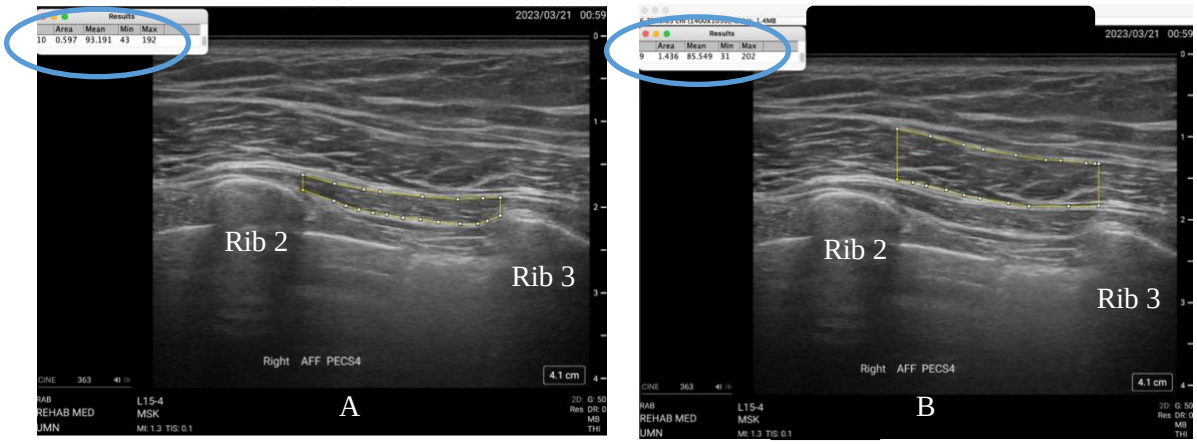

Figure 19. Pectoralis minor measurement (A) and pectoralis major measurement (B) using the polygon function. The Results table in the top left portion of the figures circled in blue display the cross-sectional area ('Area', in cm^2), and the mean echogenicity based on gray scale analysis ('Mean').

The ROI of the SA was defined by the superior and inferior boundaries of the outermost edges of the cortex of the inferior border of the 5th rib and the superior border of the 6th rib respectively. Superficial and deep boundaries excluded adjacent bone and fascial borders of each muscle, clearly delineated by the white appearance of the epimysium at the perimeter of the muscle. See Figure 20.

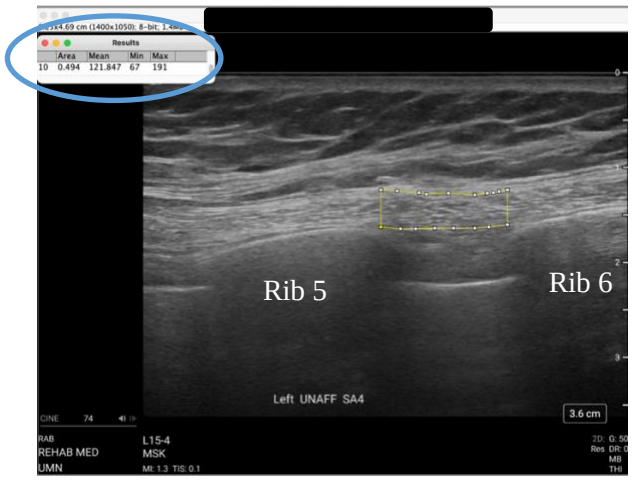

Figure 20. Serratus anterior measurement using the polygon function with the region of interest between ribs 5 and 6. The Results table demonstrates the cross-sectional area ('Area', in cm), and the mean echogenicity based on gray scale analysis ('Mean').

Kinematic Data Handling and Reduction

Specific shoulder joint motions (ST, GH, and humerothoracic) were collected by FoB and analyzed as per Wu et al (CITE) by MotionMonitor Software. Scapulothoracic rotations are described using a Cardan sequence (Y-X'-Z"): first about the Y-axis into internal rotation (+) or external rotation (-), then about the X-axis into downward rotation (+) or upward rotation (-), and then about the Z-axis into posterior tilt (+) or anterior tilt (-). Glenohumeral rotations are described using Y-X'-Z" Cardan angles referencing elevation plane (sagittal, coronal, or midway between sagittal and coronal), elevation angle, and internal (+) or external rotation (-). Humerothoracic rotations are be described using Euler angles Y-X'-Y", or elevation plane (flexion or abduction), elevation angle, and internal/external rotation respectively.(47)

The above coordinate systems are correct when testing the right side. For left-sided data collection, axis orientation (positive or negative value) for scapular internal rotation and posterior tilt as well as clavicle retraction and posterior rotation were reversed. For example, scapula internal rotation is positive on the right and negative on the left. The left-handed scapula internal rotation values were multiplied by negative one to adjust for these discrepancies. The same adjustments were made for ST posterior tilt, SC retraction and SC posterior rotation data. Separate post-processing was performed in MATLAB® (2019) for clavicle posterior rotation to correctly align the z-axis to the thorax z-axis due to potential jig-related errors.

Kinematic data were imported from MotionMonitor software into excel (Version 16.73, Microsoft Corporation, Redman, WA). Data were analyzed at 30°, 60°, 90°, and 120° of humerothoracic elevation as kinematic variables vary among symptomatic and asymptomatic subjects at different angles of elevation (47,48,83). Kinematic data at angles less than 30°, i.e. at rest and at 0° of humerothoracic elevation, were not evaluated as trunk angle can vary between subjects (47). Additionally, data at angles greater than 120° of humerothoracic elevation were not evaluated as scapular rotations have large errors using the chosen acromion method above 120° (46).

Radiation CT Scan Data Handling and Reduction

The CT scans were transferred onto Velocity™ software (Varian Medical Programs, Palo Alto, CA) by Dr. Yuan and Shane Edlund, M Health Fairview Certified Medical Dosimetrist. CT scans were taken either using a deep inspirational breath hold or free breathing technique. Treatment

plans were generated for the breast with or without regional lymph nodes and separately for the lumpectomy cavity boost. Shane Edlund merged the plans by fusing the scan images as closely as possible, and then uploading the combined scans along with the dose information to Velocity™.

RAB received pilot training in Velocity™ software from the Radiation Oncology staff prior to study initiation, then utilized Velocity™ to contour each muscle of interest within the radiation field. Anatomy was validated with guidance from Dr. Yuan and other radiologists at the University of Minnesota, in addition to verification with IMAIOS Inc. (c/o Orbiss Inc, New York, New York), a medical imaging and e-learning tool. The PMaj, PMin, and SA were individually contoured in each 3mm axial image from the inferior margin of the cricoid process to the inferior edge of the planned target volume. See Figure 21.

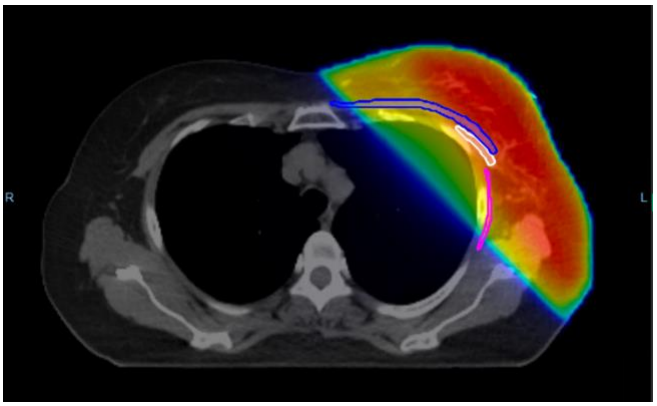

Figure 21. Computed tomography simulation scan for a participant who had radiation therapy delivered as breast tangents (4240 cGy) and a tumor bed boost (1000 cGy) for a total of 5240 cGy. Pectoralis major is outlined in dark blue, pectoralis minor in white, and serratus anterior in pink. Radiation intensity is signaled by colorwash, with red representing near maximal radiation (5240cGy) and lighter colors representing less radiation exposure.

After individual muscle segmentation, radiation dose to each muscle was calculated using Velocity™. Basic outcome measures included max dose, min dose, and mean dose for each muscle. A secondary DVH was also prepared for each muscle using Velocity™ that included exposure (Gy) on the x-axis and percent of muscle volume exposed to that dose on the y-axis. See Figure 22. The DVH calculates percentage of these selected muscle volumes that were exposed to specific radiation doses, e.g. the percentage of each muscle receiving at least 10 Gy (V10), 15 Gy (V15),

30Gy (V30), and 40Gy (V40). The doses utilized in this analysis were chosen based on existing literature as potential levels at which significant tissue damage may occur (25,74,200).

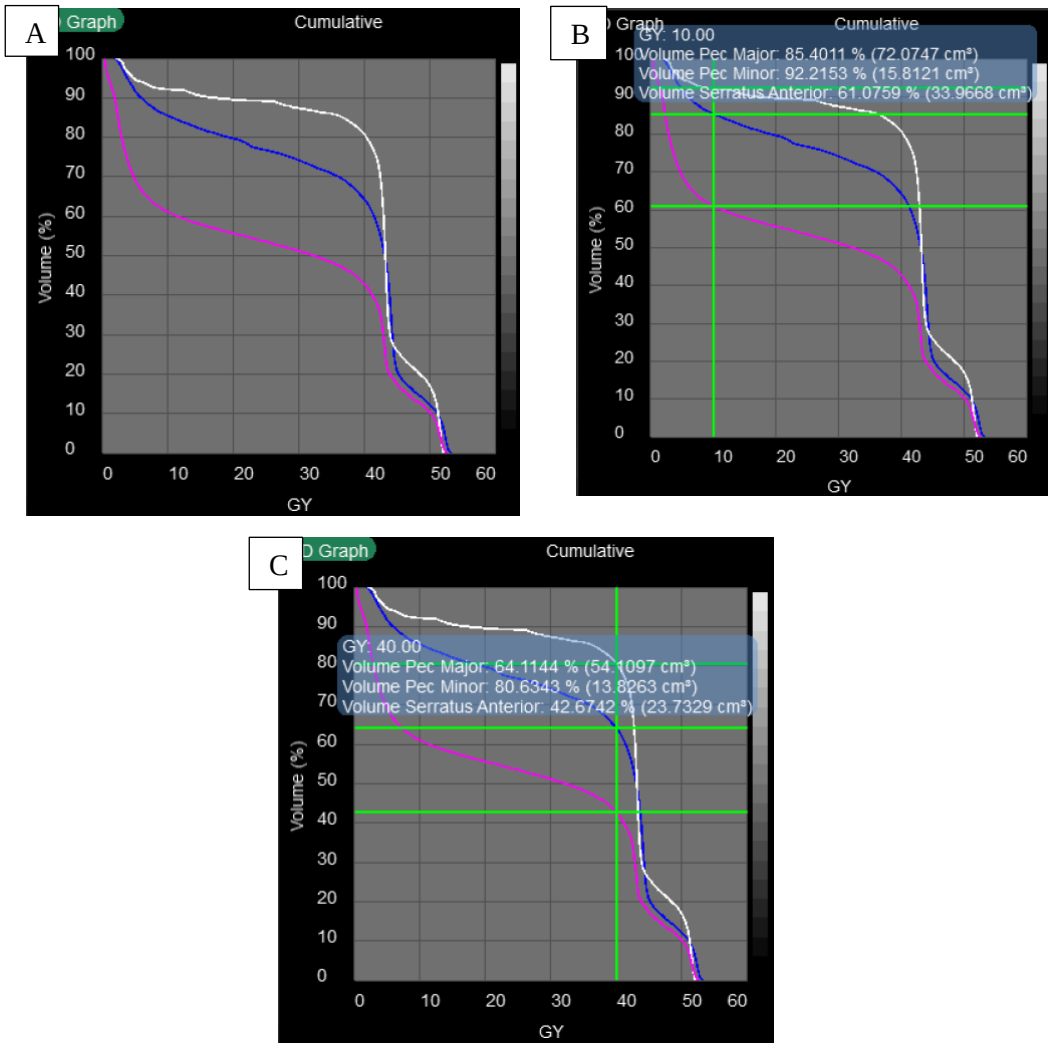

Figure 22: Dose Volume Histograms (DVHs). (A) DVH with pectoralis minor (white), pectoralis major (blue) and serratus anterior (pink) outlined. (B) V10 (percent of each muscle that received at least 40 Gy and (C) V40 (percent of each muscle that received at least 40 Gray).

Questionnaire Data Handling and Reduction

All questionnaire data was collected in REDCap and downloaded to Microsoft Excel (Version 16.73, Microsoft Corporation, Redman, WA) in Box Secure Storage. Regarding the participant questionnaire for breast cancer survivors, questions with multiple options or multiple answers (i.e. race, symptoms, etc.) were coded numerically by RAB to prepare for statistical analysis. In cases

where data was collected in both EPIC medical records and in the questionnaire (e.g. use of hormone blockers), EPIC data was utilized for accuracy of data analysis and reporting.

The PSS was entered into an Excel scoring sheet to calculate pain, satisfaction, and function scores. The pain score was calculated from 3 questions: pain at rest, pain with normal activity, and pain with strenuous activity. The participant rated their pain in each category from 0 (no pain) to 10 (worst pain possible). The pain score was the sum of those three questions. The satisfaction score was from one question, 'how satisfied are you with the current level of function of your shoulder' and again, the participant scored from 0 (dissatisfied) to 10 (very satisfied). The function score is calculated from 20 questions regarding shoulder function. Participants rated their ability to perform specific activities from 0 (can't do at all) to 3 (no difficulty) with an option to mark if they did not do that activity before their injury. The function score was calculated taking into account those questions that the participant was not able to do prior to injury. The total PSS score was the sum of all 3 subscores, with a max score of 100 reflecting maximal function and satisfaction and no pain (206). See Appendix A.

CHAPTER 4: STATISTICAL ANALYSES

Aim 4 (US reliability study in healthy controls)

Intra-class correlation coefficients (ICC 3,1) and standard error of the measurement (SEM) were obtained for EI and CSA of the PMaj, PMin, and SA using ANOVA analysis embedded in SPSS (SPSS Version 28.0.1.1) and analyzed using the mixed model, single rater, absolute method to quantify how much the repeated measures agreed with each other using RAB only as the single rater (56). Three ultrasound scans were used for reliability study analyses. Three images were chosen at random if 4 or more images were taken. The equation ICC (3,1) utilizes the following equation as per Koo et al (56) :

(MS(row) – MS (error))/(MS(row) + (k-1)(MS(error)) + k/n (MScolumn-MSerror)

(where MS = mean square, k is number of measurements (3) for each muscle, and n=number of participants)

Aim 2 (US data: EI and CSA, breast cancer survivors)

Means and standard deviations were calculated for each EI and CSA by treatment side and dominant side. Wilcoxon rank tests with *p*-values were used to determine if there was a statistical

difference between mean outcome across treatment sides within the dominant side and nondominant side separately. Spearman correlations were calculated to look for patterns in the outcome measurements within EI or CSA. Outcome data were investigated to ensure normality using histograms and Shapiro-Wilk Test; removing outliers, influential points, and transforming data as needed. Seven outliers were identified and removed for the ultrasound skeletal muscle morphology data set (1 in PMaj EI, 3 in PMin CSA, and 3 in SA CSA). Serratus anterior CSA was log transformed to be normal. Analysis was consistent with and without including outliers.

Multiple regression models were used to assess relationships between RT and each of the US measurements included in the model several covariates (chemotherapy, endocrine therapy, age at time of surgery, age at end of RT, time since RT ended, BMI, RT breast fraction dose, RLNR, and total number of RT fractions). All predictor variables are adjusted for other predictors in the model, *p*-value set at <0.05 unadjusted for multiple testing and *q*-value represented the adjusted *p*-value with significance also set at <0.05. Forest plots were used to display scale model estimates for each covariate. We used R [version 4.3.1] for all analyses and a p-value cutoff <0.05 to determine significance with a q-value (false discovery rate) used for multiple testing correction in the regression models.

Aim 1 (kinematic analysis, breast cancer survivors)

All statistical analyses for Aims 1-3 were performed in R [version 4.3.1] by a biostatistician at the Masonic Cancer Center (RJ). Means and standard deviations were calculated for each 3D kinematic outcome (ST upward rotation, ST internal rotation, ST posterior tilt, SC elevation, SC retraction, and SC posterior rotation) by treatment side and by dominant side. Wilcoxon rank tests with *p*-values were used to determine if there was a statistical difference between mean outcome across treatment sides with and without considering hand dominance. Outcome data were investigated to ensure normality using histograms and Shapiro-Wilk Test, removing outliers and influential points as needed. Outliers were defined using the identify outlier function in R and boxplots, where outliers are defined as third quartile + 1.5x interquartile range (3^{rd} quartile-1^{st} quartile). A few outliers were removed from each of the following datasets, although regression was the same for all variables with and without including the outliers: ST upward rotation, ST internal rotation, ST posterior tilt, SC elevation, and SC retraction.

Multiple regression models were used to assess relationships between adjuvant RT and each of the kinematic rotation measurements included in the model using several covariates (age at end of RT, BMI, Cancer on dominant side, Total Breast + Lumpectomy Dose, Regional Lymph Node Radiation, Fraction Dose, Radiation Dermatitis Grade/ Acute Toxicity Profile, Total # Fractions, Radiation Therapy, Endocrine Therapy, Time since RT, Chemo + Endocrine Therapies). All predictor variables are adjusted for other predictors in the model. β is a regression coefficient which represents the degree of change in the dependent/outcome variable for every 1-unit change in the predictor variable. The *p*-value for all regressions was set at <0.05 but was unadjusted for multiple testing. Therefore, the *q*-value represented the adjusted *p*-value to account for false discovery rate with significance also set at <0.05.

Aim 3 (PSS analysis)

Hypothesis 3.1

Multiple regression models were used to assess relationships between PSS and RT including several covariates. Model 1 included SA mean dose and V30 value, tumor location/breast quadrant, and total RT dose. Model 2 included PMaj and PMin mean dose and V30 value as well as RLNR and total RT dose. Model 3 included total RT dose, boost dose, and RLNR. All models corrected for other variables included in that model. The *p*-value was set at <0.05 unadjusted for multiple testing and *q*-value represented the adjusted *p*-value with significance also set at <0.05. Forest plots were used to display scale model estimates for each covariate. Outcome data were investigated to ensure normality using histograms and Shapiro-Wilk Test; removing outliers, influential points, and transforming data as needed.

Hypothesis 3.2

A Spearman rank correlation coefficient was computed in R as above to assess the relationship between PSS scores and EI data. PSS variables included PSS total as well as the 3 subscales: PSS function, PSS pain, and PSS satisfaction. Significance was set at $p < 0.05$.

Power Analysis and Sample Size

A priori power and sample size were calculated to provide 80% power, as is commonly used for biomechanical studies, to detect differences of 6.5° (26° +/- 7.52° vs 19° +/- 3.16°) for scapular upward rotation at 60° of arm elevation in a cohort of healthy individuals with and without shoulder

pain (47). Alpha level was set at 0.05 to minimize the chance of type I error, or the likelihood of finding false positives. Sample size was estimated to be 24 participants using G-power (version 3.1, Kiel, Germany). Anticipating approximately 20% dropout, 30 volunteers were recruited.

Secondary and tertiary power analyses were performed based off data from Yang et al (2013) that examined vaginal wall fibrosis and cross-sectional area comparing controls to post-radiation also using G-power.(51) Sample size was estimated to be 24 (12 in each group) using alpha = 0.05, 80% power, 2-tailed t-test in an a-priori analysis based on CSA data. Sample size was estimated to be 18 (9 in each group) using a 2-tailed t-test and a-priori analysis using alpha = 0.05 and 80% power based on EI data.

A priori power and sample size were calculated for Aim 4 (US intra-rater reliability) separately also using G-power. A sample size of 5 was needed to provide 80% power to detect an ICC of 0.96 or higher with 5% two-sided type I error rate.

CHAPTER 5: RESULTS

Aim 4: Healthy Cohort – US Reliability Study Demographics

Five healthy females between the ages of 33 and 63 with BMI 18.5 – 31.0 participated in the ultrasound reliability study. Four of the women were Caucasian and one was Asian. See Table 1.

Table 1: Demographics of Healthy Cohort

Characteristics		
Age (years)	Mean (SD)	52.3 (12.6)
	Range	33.4 – 63.9
BMI (kg/m^2)	Mean (SD)	23.1 (5.0)
	Range	18.5 – 31.0
Race	White n (%)	4 (80%)
	Other (Asian) n (%)	1 (20%)

SD = standard deviation, BMI = body mass index

ICC (3,1) for CSA of the PMaj, PMin, and SA were 0.89, 0.86, and 0.18 respectively. ICC for EI of the PMaj, PMin, and SA were 0.92, 0.82, and 0.68, respectively. See Table 2. Therefore, PMaj and PMin CSA and EI ICCs were indicative of good reliability as defined by Portney and Watkins (216) but SA CSA and EI had poor and moderate reliability, respectively.

Table 2: Ultrasound reliability results for the pectoralis major, pectoralis minor, and serratus anterior echo intensity and cross-sectional area in the healthy cohort.

		Mean (SD)	ICC (95% CI)	SEM[3]
Pectoralis Major	EI [a]	75.71 (21.44)	.92 (.78, .98)*	7.03
	CSA[b]	1.55 (0.55)	.89 (.72,.97)*	0.21
Pectoralis Minor	EI	77.42 (20.81)	.82 (.58, .95)*	7.33
	CSA	0.88 (0.51)	.86 (.65, .96)*	0.20
Serratus Anterior	EI	85.68 (19.15)	.68 (.27, .90)	25.58
	CSA	0.45 (0.27)	.18 (-.17, .63)	0.14

[1] EI = echo intensity (in grayscale level); [2] CSA = cross-sectional area (cm^2), [3] SEM=standard error of measurement calculated as the square root of the mean square error from ANOVA.
*Significant at $p<0.001$

Aims 1-3: Breast Cancer Survivors – Demographics, Oncology-Related Characteristics

Thirty breast cancer survivors were recruited to participate in the study between 09/2022 and 04/2023. Four consented participants were not included in the final study (dropout = 13.3%). One participant had an injury to her affected shoulder the week before the study was conducted which RAB was not aware of until the day of the study. One participant dropped out due to pregnancy between the time of consent and day of study. One had an ALND which is an exclusion criterion and was missed at the time of screening. The fourth was consented but failed to schedule.

Therefore, the final number of breast cancer survivors that participated in this study was 26. The average age of breast cancer survivors was 62.2 +/- 7.5 years, and their mean age at the time of radiation was 56.9 +/- 7.0 years (range 40 – 68 years of age). Their mean BMI at the time of radiation was 27.0 (range 18.9 – 36.8). Ninety-five percent of the participants were White, and 5% were Korean. See Table 3.

Oncologic characteristics of the breast cancer cohort are shown in Table 4. All participants had a lumpectomy and sentinel lymph node biopsy. The majority of breast cancer survivors were diagnosed with Stage 1 cancer (62%) with the remainder diagnosed with Stage 2 cancer (38%). No participants in this study were diagnosed with Stage 3 or Stage 4 cancer. Nineteen participants did not have chemotherapy (73%), with 7 participants (27%) having either neoadjuvant or adjuvant chemotherapy. Many participants were on hormone blocking medication (77%) due to having

hormone positive breast cancers. Only 12% of participants had both endocrine therapy and chemotherapy.

Table 3: Demographics of Breast Cancer Survivors (n=26)

Characteristics		**Mean (SD) or n (%)**
Age at time of study (years)	Mean (SD)	62.2 (7.5)
	Range	42 - 72
Age at time of surgery (years)	Mean (SD)	56.6 (7.2)
	Range	40 – 68
Age at end of radiation (years)	Mean (SD)	56.9 (7.0)
	Range	40 - 68
BMI at time of surgery (kg/m^2)	Mean (SD)	27.2 (5.9)
	Range	18.9 – 40.9
Race	White	25 (96%)
	Other (Korean)	1 (4%)
Diabetes	Yes	0 (0%)
	No	26 (100%)

Table 4: Oncologic Characteristics of Breast Cancer Survivors

Characteristics		**Mean (SD), median (range), or n (%)**
Breast Surgery Type	Lumpectomy	26 (100%)
	Mastectomy	0 (0%)
Lymph Node Surgery Type	Sentinel Lymph Node Biopsy	26 (100%)
	Axillary Lymph Node Dissection	0 (0%)
Lymph Node Surgery Details	Number of lymph nodes removed	2 (1 - 5)
	Number of lymph nodes positive	1 (0 - 2)
Cancer Stage	1	16 (62%)
	2	10 (38%)
	3	0 (0%)
	4	0 (0%)
Cancer Subtype	Estrogen and/or progesterone-receptor positive	22 (85%)
	Her2 (+)	2 (8%)
	Triple Negative	2 (8%)
Laterality of Cancer	Right	13 (50%)
	Left	13 (50%)
Cancer on Dominant Side	Yes	12 (46%)
	No	14 (54%)
Chemotherapy (n,%)	None	19 (73%)
	Neoadvjuvant (preoperative) chemotherapy	3 (12%)
	Adjuvant (postoperative) chemotherapy	4 (15%)

Radiation	Total radiation dose (cGy) (median, range)	5240 (3200 - 6290)
	Lumpectomy cavity boost (number of participants)	19 (73%)
	Regional lymph node radiation[1] (number of participants)	3 (12%)
	Conventionally fractionated radiation therapy (number of participants)	5 (19%)
	Hypofractionated radiation therapy[2] (number of participants)	20 (77%)
	Time between end of radiation and study testing (months)	54 (26)
	Radiation Dermatitis Grade / Acute Toxicity Profile by CTC AE4[2] (n, %)	Grade 1: 16 (62%) Grade 2: 6 (23%) Grade 3: 2 (8%) Unknown:[4] 1 (4%)
Endocrine Therapy	Yes	20 (77%)
	No	6 (23%)
Chemotherapy + Endocrine therapy	Yes	3 (12%)

[1] 2 participants had supraclavicular lymph node radiation only, 1 participant had supraclavicular plus axillary lymph node radiation
[2] Hypofractionated radiation therapy was defined as greater than or equal to 2.0Gy per fraction
[3]CTC AE4 is Common Terminology Criteria for Adverse Events (CTCAE) v4.0 as diagnosed by the Radiation Oncologist at the end of radiation treatment.
[4] Not known, treated by radiation oncologist outside M Health Fairview

A custom questionnaire was developed to capture patient-reported symptoms following adjuvant RT. The results of this questionnaire are listed below in Table 5.

Table 5. Participant self-report of symptoms experienced during or after radiation therapy

	Yes	No
Skin irritation in radiation field during or after radiation	20 (76.9%)	6 (23.1%)
If radiation symptoms, how would you describe your symptoms?		
Red	17 (65.4%)	9 (34.6%)
Itchy	12 (46.2%)	14 (53.8%)
Rash / bumpy	7 (26.9%)	19 (73.1%)
Blister	4 (15.4%)	22 (84.6%)
My skin peeled	5 (19.2%)	21 (80.8%)
Sunburn	10 (38.5%)	16 (61.5%)
Have you had any current or past problems with your affected arm, shoulder, or chest wall?	11 (42.3%)	15 (57.7%)
Discomfort / Pain	6 (23.1%)	20 (76.9%)
Stiffness	10 (38.5%)	16 (61.5%)

Tightness	8 (30.8%)	18 (69.2%)
Weakness	6 (23.1%)	20 (76.9%)
Achiness	8 (30.8%)	18 (69.2%)
Heaviness	3 (11.5%)	23 (88.5%)
Fullness	2 (7.7%)	24 (92.3%)
Swelling	3 (11.5%)	23 (88.5%)
Numbness / Tingling	2 (7.7%)	24 (92.3%)
Pulling	6 (23.1%)	20 (76.9%)
Stinging	4 (15.4%)	22 (84.5%)
Stabbing	4 (15.4%)	22 (84.5%)
Tenderness	5 (19.2%)	21 (80.8%)
Lymphedema	8 (30.8%)	18 (69.2%)
Axillary web syndrome	3 (11.5%)	23 (88.5%)
# of participants with at least 4 symptoms listed above	9 (81.8%)	2 (18.2%)
Number of participants with arm, shoulder, or chest wall symptoms within 1 week of the study	11 (42.3%)	15 (57.7%)
If symptoms were felt, where were they felt?		
Shoulder	10 (38.5%)	16 (61.5%)
Chest wall	5 (19.2%)	21 (80.8%)
Breast	2 (7.7%)	24 (92.3%)
Arm	7 (26.9%)	19 (73.1%)
Elbow	5 (19.2%)	21 (80.8%)
Wrist	0 (0%)	26 (100%)
Hand	0 (0%)	26 (100%)
Fingers	0 (0%)	26 (100%)
Neck	2 (7.7%)	24 (92.3%)
Axilla	1 (3.8%)	25 (96.2%)
Back / nerves in radiation field	1 (3.8%)	25 (96.2%)
What started your symptoms?		
Cancer Surgery	5 (19.2%)	21 (80.8%)
Radiation	8 (30.8%)	18 (69.2%)
Trauma	0 (0%)	26 (100%)
I don’t know / Other	3 (11.5%)	23 (88.5%)
How long after radiation ended did your symptoms begin?		
Immediately	3 (11.5%)	23 (88.5%)
< 3 months after	4 (15.4%)	22 (84.5%)
3-6 months after	0 (0%)	26 (100%)
6-9 months after	0 (0%)	26 (100%)
9-12 months after	0 (0%)	26 (100%)
> 12 months after	2 (7.7%)	24 (92.3%)
I am not sure	2 (7.7%)	24 (92.3%)
Have you ever been told by a provider that you have any of the following?		
Lymphedema of the arm, breast, or chest wall	8 (30.8%)	18 (69.2%)
Rotator cuff tendonitis or tear	2 (7.7%)	24 (92.3%)
Shoulder strain	1 (3.8%)	25 (96.2%)
Shoulder impingement	1 (3.8%)	25 (96.2%)

Axillary web syndrome	2 (7.7%)	24 (92.3%)
Other (patient responses included carpal tunnel syndrome, shoulder and neck pain, undiagnosed shoulder and neck pain, golfers elbow, tennis elbow)	3 (11.5%)	23 (88.5%)
Number of participants who had physical therapy	5 (19.2%)	21 (80.8%)
Number of participants who had lymphedema therapy	1 (3.8%)	25 (96.2%)

Aim 2 Results (Ultrasound, survivors)

Echo Intensity

Mean values: Average values for EI for each muscle studied are shown in Table 7. No statistically significant difference in means was found between the treated and untreated side for any of the three muscles examined when covariates were not included in the model (p-value > 0.05). The average (SD) EI for the affected vs. unaffected sides of the pectoralis major was 96 (16) vs. 92 (16), of the pectoralis minor was 90 (13) vs. 88 (13), and of the serratus anterior was 106 (19) vs 100 (18). See Table 6.

Table 6. Echogenicity results expressed as mean (standard deviation) with Wilcoxon rank sum test by treatment side.

Characteristic	Overall (n=52)[1]	Affected (n=26)[1]	Unaffected (n=26)[1]	p-value[2]
Pectoralis Major	94 (16)	96 (16)	92 (16)	0.5
Pectoralis Minor	89 (13)	90 (13)	88 (13)	> 0.9
Serratus Anterior	103 (18)	106 (19)	100 (18)	0.3

[1] Mean (standard deviation)
[2] Wilcoxon rank sum exact test

Linear Regression Analysis:

Multiple linear regression analysis was then performed to examine the effect of affected side vs unaffected side on EI of the PMaj, PMin, and SA within the same individual while accounting for potential confounders and effect modifiers. The data was originally run 2 ways: 1) affected vs unaffected between individuals, and 2) affected-unaffected within the same individual. As our dissertation had proposed the latter, we utilized the second model in the final analysis. The following variables were included in the final model as they demonstrated significance (p-value < 0.05) in at least one of these original models: demographic variables (age at time of surgery and age at end of RT, BMI), and treatment variables (endocrine therapy, chemotherapy, total radiation

dose, regional lymph node radiation, total number of radiation fractions, time since end of RT). In the model that was chosen (affected – unaffected within the same individual), none of these covariates were significant. The 'within participant' model was chosen as this was our original data analysis design. In this model, none of the covariates were significant. See Table 7.

Other potential variables were tested as well but were not found to significantly affect EI in any model (smoking, CTCAE skin reaction grade at the end of radiation, age at time of radiation, the use of hypofractionated vs conventional RT, and the use of both chemotherapy and hormone therapy) and these were excluded from the final models. AWS was proposed to be a covariate, but due to researcher error only 62% of the participants were examined for AWS. Some of those participants stated they did not have AWS, but it was found upon examination by RB therefore we could not depend on self-report as many women have AWS but are not aware of it.

Table 7. Linear regression results examining the effect of potential covariates on the average difference (affected – unaffected sides) of echo intensity for the pectoralis major, pectoralis minor, and serratus anterior. Each factor is presented as adjusting for all other factors in the same model.

	Pectoralis Major		Pectoralis Minor		Serratus Anterior	
	β (95%CI)	*p-value*	β (95%CI)	*p-value*	β (95%CI)	*p-value*
Endocrine therapy	-3.7 (-31, 23)	0.8	-13 (-36, 11)	0.3	5.1 (-24, 34)	0.7
Chemotherapy	-7.6 (-29, 14)	0.5	-17 (-35, 2.2)	0.08	-7.0 (-30, 16)	0.5
Age at time of surgery	-7.0 (-24, 10)	0.4	1.3 (-14, 16)	0.9	7.7 (-11, 26)	0.4
Age at end of radiation therapy	7.1 (-11, 25)	0.4	-1.4 (-17, 14)	0.9	-7.7 (-27, 12)	0.4
Body mass index	0.30 (-0.88, 1.5)	0.6	-0.09 (-1.1, 0.94)	0.9	-0.05 (-1.3, 1.2)	>0.9
Fraction dose	0.08 (-0.09, 0.26)	0.3	-0.01(-0.16, 0.14)	0.3	0.093 (-0.16, 0.14)	>0.9

Regional lymph node radiation	5.6 (-29, 18)	0.6	-5.3 (-26, 15)	0.6	-17 (-43, 7.9)	0.2
Total number of radiation fractions	0.93 (-1.3, 3.1)	0.4	0.07 (-1.9, 2.0)	>0.9	1.0 (-1.4, 3.4)	0.4
Time since radiation ended (months)	-0.20 (-0.59, 0.19)	0.3	0.04 (-0.3, 0.38)	0.8	-0.07 (-0.49, 0.35)	0.7

*Data expressed as β (95% CI), *p*-value. *q*-values (adjusted for multiple testing) for all results were $\geq$ 0.8.

Cross Sectional Area

Average values for CSA of each muscle studied are shown in Table 8. No statistically significant difference in mean CSA was found between the treated and untreated sides within all muscle groups (p-value > 0.05). The CSA of the affected vs. unaffected sides of the pectoralis major (expressed as mean (SD)) was 1.77 (0.57) vs 1.75 (0.56), of the pectoralis minor was 1.26 (0.60) vs 1.46 (0.69), and of the serratus anterior was 0.86 (0.39) vs 0.80 (0.33).

Table 8. Cross sectional area results expressed as mean (standard deviation) with Wilcoxon rank sum test by treatment side.

Characteristic	Overall (n=52)[1]	Affected (n=26)[1]	Unaffected (n=26)[1]	p-value[2]
Pectoralis Major	1.76 (0.56)	1.77 (0.57)	1.75 (0.56)	> 0.9
Pectoralis Minor	1.36 (0.65)	1.26 (0.60)	1.46 (0.69)	0.3
Serratus Anterior	0.83 (0.36)	0.86 (0.39)	0.80 (0.33)	0.8

[1] Mean (standard deviation)

[2] Wilcoxon rank sum exact test

Regression analysis:

The same potential covariates used in the EI analysis were used in the CSA multiple linear regression analysis . Again, the primary objective was to determine if mean CSA measurement was different between the affected and unaffected sides within the same individual while including potential confounders and effect modifiers in the model.

No covariates had a significant ($p < 0.05$) effect on CSA of any of the muscles examined. However, there was a trend for those women who had endocrine therapy to have slightly increased CSA of the PMaj ($p = 0.058$) and PMin ($p = 0.051$). In other words, those who had endocrine therapy had on average 1 cm^2 greater difference (β = affected – unaffected sides) in CSA of the PMaj when comparing the affected – unaffected sides compared to those who did not have endocrine therapy. Similarly, those who had endocrine therapy had on average a 1.1 cm^2 (β = affected – unaffected) greater difference in CSA of the PMin when comparing the affected – unaffected sides compared to those who did not have endocrine therapy. See Table 9 below.

Table 9. Linear regression results examining the effect of potential covariates on the average difference (affected – unaffected sides) of CSA for the pectoralis major, pectoralis minor, and serratus anterior muscles.

	Pectoralis Major		Pectoralis Minor		Serratus Anterior	
	β (95%CI)	*p-value*	β (95%CI)	*p-value*	β (95%CI)	*p-value*
Endocrine therapy	1.0 (-0.04, 2.1)	0.06	1.1 (-0.01, 2.1)	.05	-0.16 (-1.1, 0.78)	0.7
Chemotherapy	0.74 (-0.11, 1.6)	0.08	0.23 (-0.62, 1.1)	0.6	0.07 (-0.68, 0.82)	0.5
Age at time of surgery	0.30 (-0.38, 0.98)	0.4	0.54 (-0.14, 1.2)	0.1	0.10 (-0.50, 0.70)	0.7
Age at end of radiation therapy	-0.29 (-1.0, 0.42)	0.4	-0.56 (-1.3, 0.15)	0.1	-0.10 (-0.73, 0.52)	0.7
Body mass index	-0.04 (-0.09, 0.00)	0.07	0.00 (-0.05, 0.4)	0.8	0.01 (-0.03, 0.05)	0.6
Fraction dose	0.00 (-0.01, 0.00)	0.3	0.00 (-0.01, 0.01)	0.7	0.00 (-0.01 0.01)	>0.9
Regional lymph node radiation	0.15 (-0.77, 1.1)	0.7	-0.70 (-1.6, 0.22)	0.13	-0.09 (-0.91, 0.72)	0.8
Total number of radiation fractions	-0.07 (-0.16, 0.02)	0.11	0.02 (-0.07, 0.11)	0.6	0.09 (-0.72, 0.91)	>0.9

Time since radiation ended (months)	0.00 (-0.01, 0.02)	0.8	0.00 (-0.01, 0.02)	0.8	0.00 (-0.01 0.01)	0.9

* p-values 0.051 – 0.058 suggestive of possible trends in the data. All data expressed as β (95% CI), p-value. q-value for all analyses was $\geq$ 0.8.

Correlations

An exploratory correlation analysis was also run to determine if EI and CSA measurements were significantly related. We saw some significant correlations within EI and CSA measurements, but the correlations of EI to CSA were all weak at $\leq$ 0.33 (not shown), suggesting that there was a very weak non-statistically significant relationship between the 2 groups of measurements. Significant correlations were found for EI between the affected muscles (i.e. affected PMaj to affected PMin, or affected PMin to affected SA, r = 0.53 – 0.76) and also between the affected and unaffected muscles (i.e. pec major of the affected to pec major of the unaffected sides, r = 0.43 – 0.63). See Table 10 for correlation results.

Table 10. Correlations between the echo intensity of the pectoralis major, pectoralis minor, and serratus anterior of the affected and unaffected sides.

	Aff Pec Major EI	Aff Pec Minor EI	Aff SA EI	Unaff Pec Major EI	Unaff Pec Minor EI	Unaff SA EI
Aff Pec Major EI		0.53*	0.76*	0.49*	0.20	0.61*
Aff Pec Minor EI			0.43*	0.46*	0.58*	0.89
Aff SA EI				0.60*	0.27	0.74*
Unaff pec major EI					0.63*	0.66*
Unaff pec minor EI						0.20
Unaff SA EI						

*p < 0.05

CSA = cross sectional area. EI = echo intensity. Pec Major = pectoralis major. Pec Minor = pectoralis minor. SA = serratus anterior. Aff = affected side. Unaff = unaffected side.

The CSA correlations among the affected muscles ranged from 0.63 (affected pec major CSA to affected pec minor CSA) to 0.14 (affected pec minor CSA to affected SA CSA). Correlations

between the affected and unaffected sides of the same muscles ranged from 0.36 (pec major) to 0.54 (pec minor). See Table 11.

Table 11. Correlations between the cross-sectional area of the pectoralis major, pectoralis minor, and serratus anterior of the affected and unaffected sides.

	Aff Pec Major CSA	Aff Pec Minor CSA	Aff SA CSA	Unaff Pec Major CSA	Unaff Pec Minor CSA	Unaff SA CSA
Aff Pec Major CSA		0.63*	0.13	0.36	0.36	0.31*
Aff Pec Minor CSA			0.14	0.29	0.54*	0.35*
Aff SA CSA				0.47*	0.60*	0.53*
Unaff pec major CSA					0.52*	0.42*
Unaff pec minor CSA						0.73*

*$p < 0.05$
CSA = cross sectional area. EI = echo intensity. Pec Major = pectoralis major. Pec Minor = pectoralis minor. SA = serratus anterior. Aff = affected side. Unaff = unaffected side.

Specific Aim 1 Results (3D Shoulder Kinematics in Breast Cancer Survivors)

Scapulothoracic upward rotation

No statistically significant ($p<0.05$) mean differences (affected – unaffected sides) were noted in the amount of ST upward rotation across HT angles. See Table 12 and Figure 23. When cancer was on the dominant side, there was a greater difference in scapula upward rotation between the affected and unaffected sides than when cancer was on the nondominant side at rest, 30° degrees abduction, and 30°, 60°, 90°, and 120° forward flexion. This suggests the differences are due to dominance and not to adjuvant RT. See Table 13. Adjustment for potential covariates (age at the end of RT, BMI, total RT dose, RLNR, whole breast fraction dose, radiation dermatitis grade, total number of radiation fractions, conventional vs hypofractionated RT, endocrine therapy, time since RT ended, chemotherapy + endocrine therapies) did not change the significance of these findings.

Table 12. Scapulothoracic upward rotation (in degrees) on the affected side vs the unaffected side during abduction and forward flexion, expressed at specific angles and amount moved between those specific angles.

Humerothoracic Angle	**Affected,** n=26[1]	**Unaffected,** n=26[1]	***p*-value[2]**
Rest (abduction and forward flexion)	-5 (9)	-4 (15)	>0.9
30° abduction	-7 (8)	-7 (15)	0.7
60° abduction	-13 (7)	-13 (15)	0.8
90° abduction	-23 (7)	-21 (15)	>0.9
120° abduction	-33 (8)	-31 (16)	0.7
30° forward flexion	-8 (9)	-7 (16)	>0.9
60 ° forward flexion	-14 (9)	-13 (16)	0.8
90 ° forward flexion	-26 (8)	-23 (17)	0.7
120° forward flexion	-35 (7)	-34 (17)	0.8
30° - 60° abduction	-6.5 (2.4)	-6.0 (3.7)	0.9
60° - 90° abduction	-9.6 (3.9)	-8.5 (2.5)	0.4
90° - 120° abduction	-9.2 (6.9)	-9.9 (3.9)	>0.9
30° - 60° forward flexion	-25 (9)	-24 (8)	0.8
60° - 90° forward flexion	-11.2 (3.3)	-10.6 (2.5)	0.4
90° - 120° forward flexion	-9.7 (5.3)	-10.7 (3.6)	>0.9
30° - 120° forward flexion	-27 (8)	-27 (6)	0.8

[1] Mean (standard deviation) in degrees

[2] Wilcoxon rank sum exact test comparing the difference of affected – unaffected of the dominant vs nondominant sides with significant *p* value set at <0.05.

Table 13. Scapulothoracic upward rotation (in degrees) on the affected and unaffected sides expressed as a function of hand dominance.

Humerothoracic Angle	**Cancer on dominant side,** n=12[1]		**Cancer on nondominant side,** n=14[1]		***p*-value[2]**
	Affected	**Unaffected**	**Affected**	**Unaffected**	
Rest (abduction and forward flexion)	-6.8 (9.4)	3.2 (16.5)	-4.3 (7.5)	-10.3 (11.5)	0.031*
30° abduction	-7.6 (8.1)	-0.86 (16.4)	-6.7 (6.7)	-11.5 (11.3)	0.041*
60° abduction	-13.4 (7.8)	-6.8 (17.8)	-13.6 (6.9)	-17.5 (10.7)	0.2
90° abduction	-22.8 (7.5)	-15.0 (17.1)	-22.8 (7.6)	-26.2 (11.5)	0.2
120° abduction	-32.6 (9.8)	-23.8 (17.5)	-33.6 (6.4)	-37.1 (12.9)	0.2
30° forward flexion	-10.1 (9.2)	-1.6 (17.4)	-7.9 (8.1)	-12.1 (12.5)	0.027*
60 ° forward flexion	-16.0 (8.6)	-7.6 (18.7)	-13.9 (8.1)	-16.9 (12.5)	0.036*
90 ° forward flexion	-27.4 (8.3)	-17.7 (19.8)	-24.5 (8.0)	-27.8 (12.8)	0.027*
120° forward flexion	-36.7 (8.3)	-26.8 (18.8)	-34.0 (7.0)	-40.0 (12.9)	0.036*

[1] Mean (Standard deviation) in degrees
[2] Wilcoxon rank sum exact test comparing the difference of affected – unaffected of the dominant vs nondominant sides
* Significant at $p < 0.05$

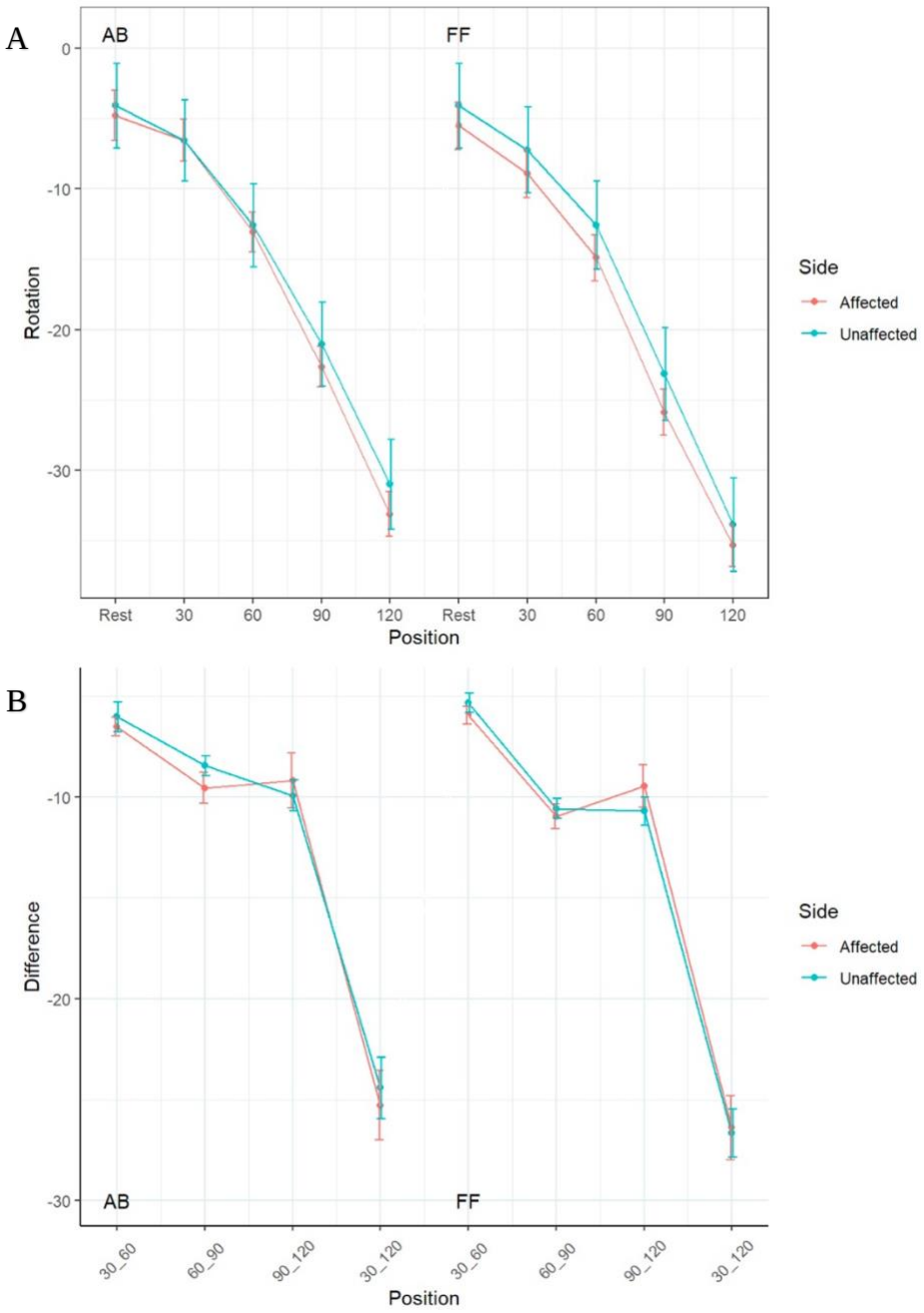

Figure 23. Scapulothoracic upward rotation (in degrees) (A) during abduction (AB) and forward flexion (FF) (top graph) and (B) the amount of scapulothoracic upward rotation that occurred between 30° - 60°, 60° - 90°, 90° - 120°, and 30° - 120° during abduction and forward flexion (bottom graph). Error bars represent standard deviation.

Scapulothoracic internal rotation

No statistically significant mean differences were found in ST internal rotation between the affected and unaffected sides at any angle. See Table 14. When cancer was on the dominant side, the affected side moved more into ST internal rotation than the unaffected side compared to when cancer was on the nondominant side between 90° - 120° of forward flexion and between 30° - 120° of forward flexion (p=0.036 and p=0.031 respectively, see Table 15). Nonsignificant trends were also noted where the affected side had more ST internal rotation throughout the range of motion than the unaffected side during both forward flexion and abduction. See Figure 24.

Table 14. Mean scapulothoracic internal rotation (in degrees) of the affected side and the unaffected side during abduction and forward flexion, expressed at specific angles and amount moved between those specific angles. Wilcoxon rank sum test p-value is also presented with p-value <0.05 used to indicate statistical significance.

Humerothoracic Angle	**Affected,** n=26[1]	**Unaffected,** n=26[1]	**p-value[2]**
Rest (abduction and forward flexion)	35 (14)	33 (14)	0.5
30° abduction	31 (14)	27 (14)	0.4
60° abduction	31 (15)	26 (15)	0.3
90° abduction	31 (16)	26 (15)	0.3
120° abduction	32 (19)	26 (16)	0.3
30° forward flexion	38 (14)	35 (14)	0.5
60 ° forward flexion	41 (14)	38 (14)	0.4
90 ° forward flexion	44 (15)	41 (15)	0.5
120° forward flexion	41 (20)	40 (18)	0.6
30° - 60° abduction	-0.15 (2.36)	-0.94 (2.10)	0.2
60° - 90° abduction	-0.3 (3.2)	-0.3 (2.8)	0.7
90° - 120° abduction	-0.3 (5.5)	-0.3 (4.8)	0.8
30° - 120° abduction	-1 (9)	-1 (9)	0.7
30° - 60° forward flexion	2.90 (1.82)	2.57 (1.85)	0.5
60° - 90° forward flexion	3.5 (2.25)	4.27 (2.82)	0.5
90° - 120° forward flexion	-2 (6)	02 (6)	0.8

30° - 120° forward flexion	4 (9)	5 (10)	>0.9

[1]Mean (standard deviation) in degrees
[2] Wilcoxon rank sum exact test

Table 15. Scapulothoracic internal rotation (in degrees) on the affected and unaffected sides expressed as a function of hand dominance.

Humerothoracic Angle	**Cancer on dominant side,** n=12[1]		**Cancer on nondominant side,** n=14[1]		***p*-value**[2]
	Affected	**Unaffected**	**Affected**	**Unaffected**	
Rest (abduction and forward flexion)	33 (15)	32 (18)	37 (15)	33 (11)	0.8
30° abduction	29 (14)	26 (18)	33 (14)	27 (12)	0.9
60° abduction	29 (14)	25 (18)	33 (15)	26 (12)	0.9
90° abduction	28 (15)	25 (17)	33 (17)	26 (14)	0.7
120° abduction	29 (16)	25 (17)	34 (22)	26 (16)	0.5
30° forward flexion	37 (14)	35 (16)	38 (15)	36 (12)	>0.9
60 ° forward flexion	40 (12)	37 (17)	41 (15)	39 (12)	>0.9
90 ° forward flexion	44 (13)	39 (16)	44 (18)	43 (14)	0.9
120° forward flexion	43 (18)	36 (17)	41 (23)	43 (19)	0.4

[1] Mean (standard deviation) in degrees
[2] Wilcoxon rank sum exact test comparing the difference of affected – unaffected of the dominant vs nondominant sides

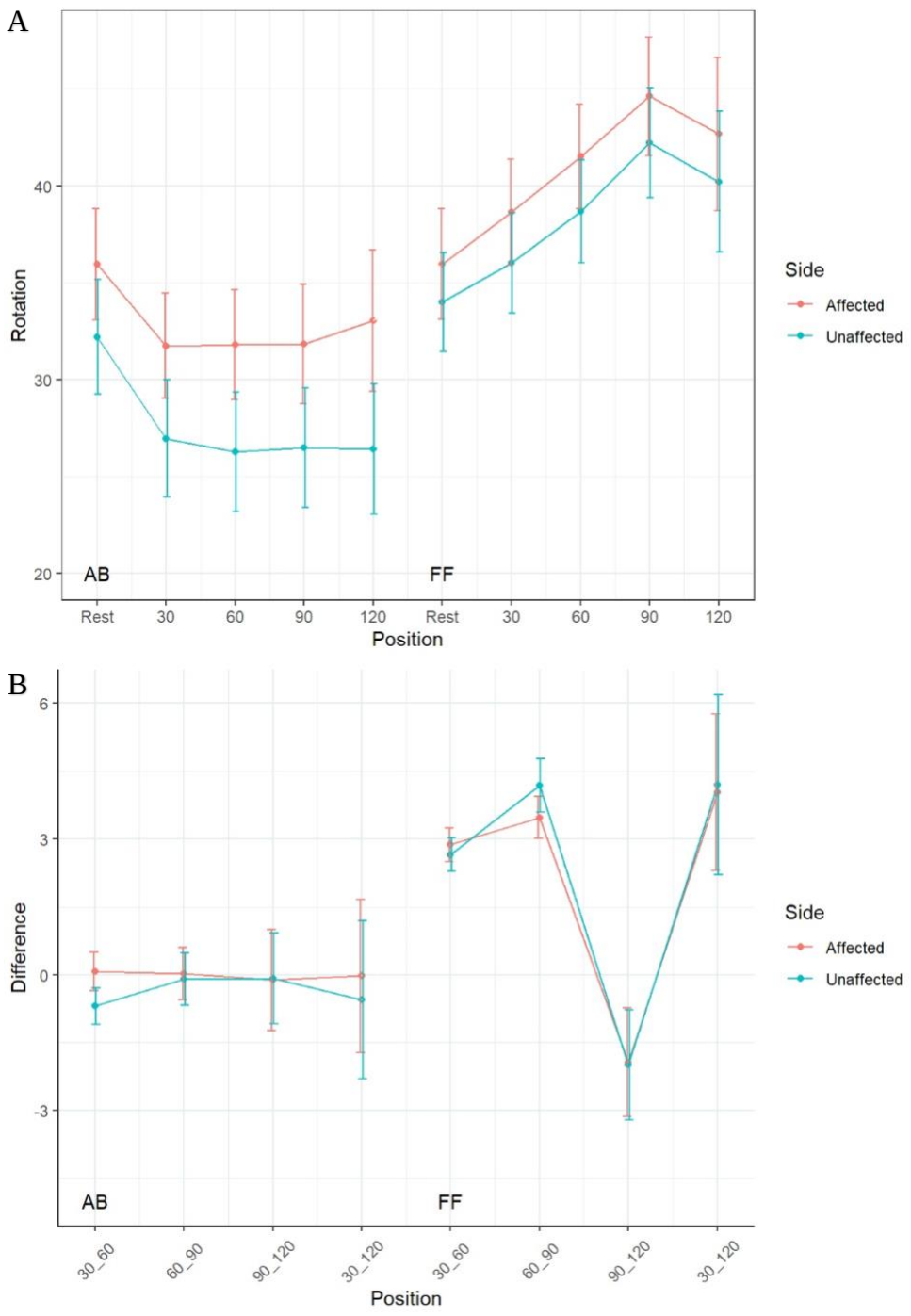

Figure 24. Scapulothoracic internal rotation (in degrees) during abduction (AB) and forward flexion (FF) (top graph) and the amount of scapulothoracic internal rotation that occurred between 30° - 60°, 60° - 90°, 90° - 120°, and 30° - 120° in abduction and forward flexion (bottom graph). Error bars represent standard deviation.

Regression analysis revealed one possible association between endocrine therapy and ST internal rotation, with those who had endocrine therapy moving almost 5° less on average than those who did not have endocrine therapy from 30° - 60° of forward flexion (β = -4.9, 95% CI = -9.1, -0.77, p=0.023). However, after adjustment for multiple testing, this was not statistically significant (q > 0.9).

Scapulothoracic posterior tilt

No statistically significant mean differences (affected – unaffected sides) were noted in ST tilt across arm elevation angles, with and without adjustment for hand dominance. See Table 16 and Figure 25 below. Data is shown for the effect of hand dominance in Appendix I.

Table 16. Mean scapulothoracic posterior tilt (in degrees) of the affected side and the unaffected side during abduction and forward flexion, expressed at specific angles and amount moved between those specific angles. Wilcoxon rank sum test p-value is also presented with p-value <0.05 used to indicate statistical significance.

Humerothoracic Angle	**Affected**, n=26[1]	**Unaffected**, n=26[1]	***p*-value**[2]
Rest	-8 (6)	-6 (10)	0.6
30° abduction	-6 (6)	-7 (12)	0.5
60° abduction	-6 (7)	-7 (11)	0.8
90° abduction	-4 (8)	-6 (12)	0.6
120° abduction	-2 (11)	-3 (15)	0.8
30° forward flexion	-6 (5)	-6 (12)	>0.9
60 ° forward flexion	-4 (5)	-4 (11)	0.7
90 ° forward flexion	-5 (8)	-5 (12)	0.7
120° forward flexion	-3 (11)	-3 (17)	0.8
30° - 60° abduction	0.04 (2.47)	0.36 (3.69)	0.7
60° - 90° abduction	1.5 (3.1)	1.1 (4.1)	0.8
90° - 120° abduction	2.1 (4.2)	2.4 (4.2)	0.7
30° - 120° abduction	4 (8)	4 (10)	>0.9
30° - 60° forward flexion	1.58 (2.34)	1.95 (3.18)	0.5

60° - 90° forward flexion	-1.0 (4.4)	-1.1 (5.4)	0.9
90° - 120° forward flexion	2.4 (5.1)	1.5 (6.3)	0.7
30° - 120° forward flexion	3 (9)	2 (12)	0.9

[1]Mean (standard deviation) in degrees
[2] Wilcoxon rank sum test

Regression analysis revealed only one statistically significant (p-value <0.05) covariate for ST posterior tilt. Those who had RLNR had on average 9.2° (β = affected – unaffected) more ST posterior tilt between 60° - 90° of forward flexion than those who did not have regional lymph node radiation after adjusting for all other factors (p = 0.021). Additionally, those who had regional lymph node radiation had on average 18° (β = affected – unaffected) more ST posterior tilt between 30° - 120° of forward flexion than those who did not have regional lymph node radiation after adjusting for all other factors (p = 0.054). However, after adjustment for multiple testing, these were not significant (q > 0.7 for both tests).

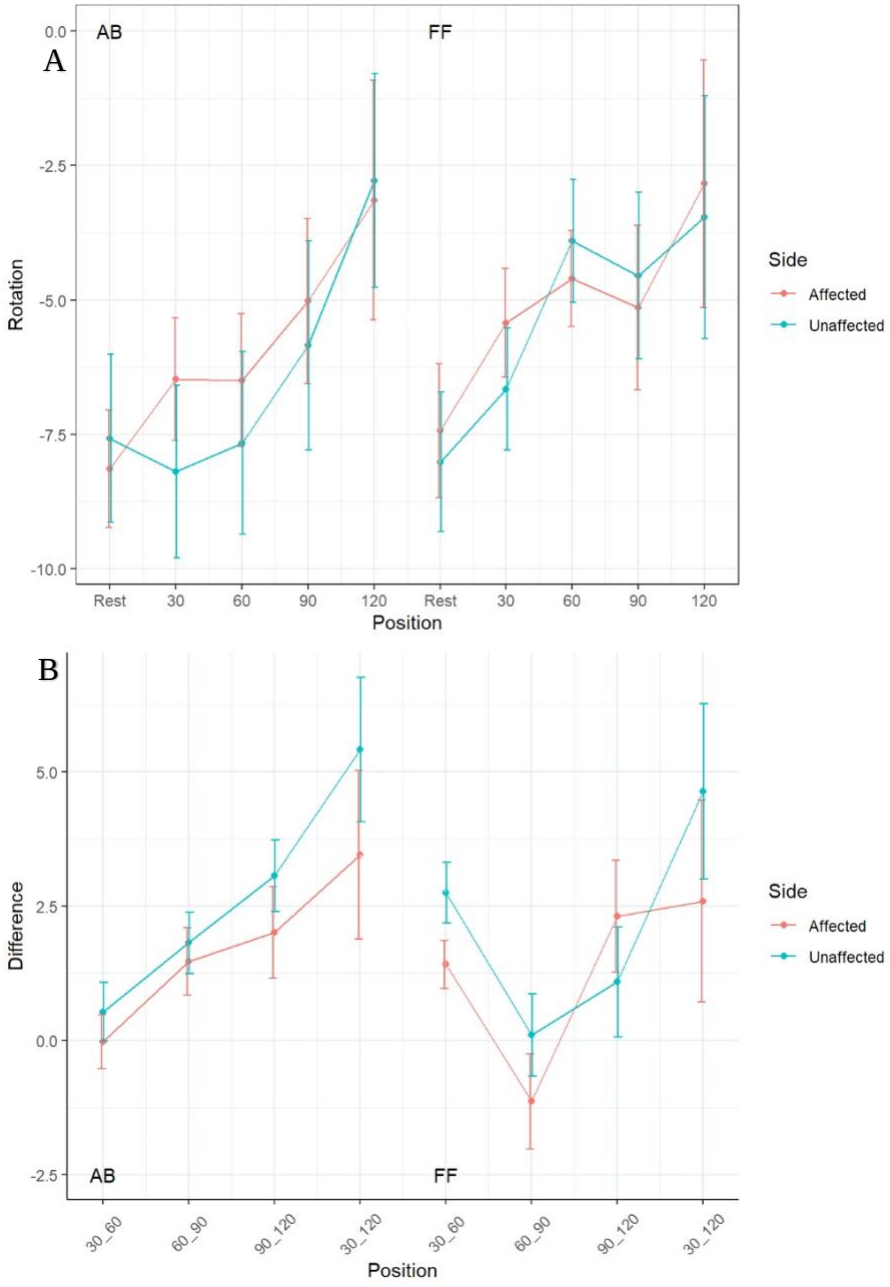

Figure 25. Scapulothoracic posterior tilt (in degrees) (A) during abduction (AB) and forward flexion (FF) and (B) the amount of scapulothoracic posterior tilt that occurred between 30° - 60°, 60° - 90°, 90° - 120°, and 30° - 120° of abduction and forward flexion. Error bars represent standard deviation.

Sternoclavicular Retraction

No statistically significant mean differences (affected – unaffected sides) were found in the amount of SC retraction across HT angles, both before and after adjustment for hand dominance. See Table 17 and Figure 26. (Hand dominance data shown in Appendix I). Regression analysis revealed one potential covariate of significance: those who had regional lymph node radiation had on average 4.2° more SC retraction (β = affected – unaffected sides) from 60° - 90° of shoulder abduction than those who did not have regional lymph node radiation after adjusting for all other factors (β = -4.2, 95% CI = -7.5, -0.92, p = 0.016) although correction for multiple testing suggests that this may not be significant (q >0.9). No other covariates significantly affected SC retraction (p > 0.05).

Table 17. Sternoclavicular retraction (in degrees) of the affected side vs the unaffected side during abduction and forward flexion, expressed at specific angles and amount moved between those specific angles.

Humerothoracic Angle	**Affected**, n=26[1]	**Unaffected**, n=26[1]	***p*-value**[2]
Rest	-20 (14)	-23 (16)	0.5
30° abduction	-24 (14)	-26 (14)	0.5
60° abduction	-27 (14)	-30 (14)	0.4
90° abduction	-30 (15)	-33 (15)	0.5
120° abduction	-34 (15)	-38 (16)	0.3
30° forward flexion	-20 (14)	-23 (14)	0.4
60 ° forward flexion	-21 (14)	-24 (14)	0.5
90 ° forward flexion	-24 (14)	-26 (14)	0.6
120° forward flexion	-30 (15)	-32 (15)	0.5
30° - 60° abduction	-3.17 (1.67)	-3.52 (1.37)	0.5
60° - 90° abduction	-3.27 (1.94)	-3.66 (1.59)	0.2
90° - 120° abduction	-4.14 (3.06)	-5.12 (2.76)	0.2
30° - 120° abduction	-11.1 (5.8)	-11.9 (5.0)	0.6
30° - 60° forward flexion	-1.29 (1.18)	-1.06 (1.02)	0.3
60° - 90° forward flexion	-2.81 (2.02)	-1.87 (1.60)	0.072
90° - 120° forward flexion	-5.70 (3.77)	-5.60 (2.82)	>0.9
30° - 120° forward flexion	-9.8 (4.8)	-8.9 (3.9)	0.4

[1]Mean (standard deviation) in degrees, [2] Wilcoxon rank sum test

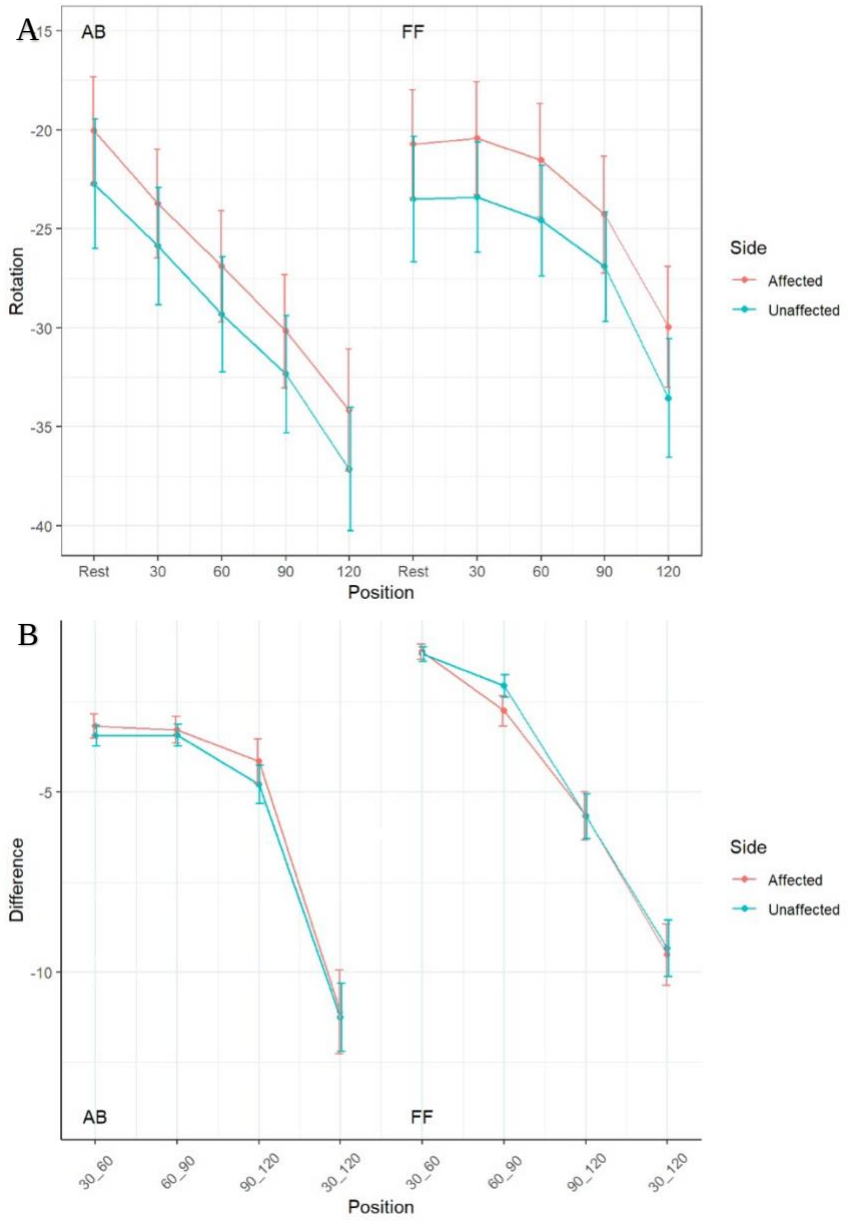

Figure 26. Sternoclavicular retraction (in degrees) during (A) abduction (AB) and forward flexion (FF) and (B) the amount of sternoclavicular retraction (in degrees) that occurred between 30° - 60°, 60° - 90°, 90° - 120°, and 30° - 120° of abduction and forward flexion. Error bars represent standard deviation.

Sternoclavicular Elevation

Statistically significant ($p<0.05$) differences (affected – unaffected sides) were found in the amount of SC elevation across HT angles at 30°, 60°, 90° of abduction and at 120° of forward flexion, with the affected side having greater SC elevation angles than the unaffected side (See Table 18). There was also a nonsignificant ($p > 0.05$) trend for SC elevation to remain higher on the affected side than the unaffected side at all angles and both planes of arm elevation. See Figure 27.

Those who had cancer on the affected side had significantly more SC elevation at 120° of forward flexion than the unaffected side ($p = 0.046$). See Table 19. However, from 90° - 120° of abduction, those who had cancer on the dominant side moved less into clavicle elevation than those who had cancer on their nondominant side ($p = 0.041$). Regression analysis revealed that those who had estrogen therapy moved 10° on average more into SC elevation from 90° - 120° of forward flexion than those who did not have endocrine therapy ($\beta = -10$, 95% CI = (-20, 0.12), $p = 0.048$) although after adjusting for multiple testing, this may not be significant ($q > 0.9$). No other covariates significantly affected SC elevation ($p > 0.05$).

Table 18. Sternoclavicular elevation (in degrees) of the affected side vs the unaffected side during abduction and forward flexion, expressed at specific angles and amount moved between those specific angles, without adjustment for hand dominance.

Humerothoracic Angle	**Affected**, n=26[1]	**Unaffected**, n=26[1]	***p*-value**[2]
Rest	-11.0 (5.2)	-8.6 (6.3)	0.14
30° abduction	-12.5 (5.0)	-9.1 (5.9)	0.014*
60° abduction	-13.7 (4.3)	-10.6 (5.7)	0.026*
90° abduction	-16.5 (4.6)	-13.8 (6.7)	0.048*
120° abduction	-21 (6)	-17 (8)	0.12
30° forward flexion	-13 (6)	-10 (7)	0.077
60 ° forward flexion	-12.7 (5.4)	-9.4 (6.2)	0.068
90 ° forward flexion	-15.2 (5.1)	-12.0 (5.9)	0.087
120° forward flexion	-21 (8)	-17 (7)	0.027*
30° - 60° abduction	-1.70 (1.46)	-1.53 (1.64)	0.7
60° - 90° abduction	-2.60 (1.50)	-2.45 (1.65)	0.7
90° - 120° abduction	-3.23 (1.97)	-3.60 (2.07)	0.6

30° - 120° abduction	-7.7 (3.9)	-7.6 (4.5)	>0.9
30° - 60° forward flexion	-0.61 (1.61)	-0.52 (1.03)	>0.9
60° - 90° forward flexion	-3.18 (2.37)	-2.56 (2.17)	0.3
90° - 120° forward flexion	-4.11 (3.24)	-4.11 (2.53)	>0.9
30° - 120° forward flexion	-7.7 (4.2)	-7.7 (4.3)	0.9

[1]Mean (standard deviation)
[2] Wilcoxon rank sum test
*Significant at $p < 0.05$

Table 19. Sternoclavicular elevation (in degrees) on the affected and unaffected sides expressed as a function of hand dominance.

Humerothoracic Angle	**Cancer on dominant side,** n=12[1]		**Cancer on nondominant side,** n=14[1]		***p*-value[2]**
	Affected	**Unaffected**	**Affected**	**Unaffected**	
Rest (abduction and forward flexion)	-12.6 (4.6)	-7.8 (7.0)	-8.4 (7.0)	-8.3 (7.1)	0.4
30° abduction	-13.4 (5.6)	-8.0 (6.9)	-10.0 (7.0)	-10.0 (9.0)	0.3
60° abduction	-15.0 (6.2)	-9.6 (7.0)	-12.0 (8.0)	-11.0 (8.0)	0.3
90° abduction	-17.5 (6.3)	-11.9 (7.0)	-15 (8)	-14 (8)	0.6
120° abduction	-21.0 (7.0)	-15.0 (8.0)	-18.0 (10.0)	-18.0 (9.0)	0.1
30° forward flexion	-14.0 (6.0)	-8.0 (7.0)	-10.0 (8.0)	-10.0 (9.0)	0.2
60 ° forward flexion	-14.0 (6.0)	-8.0 (6.0)	-11.0 (8.0)	-10.0 (9.0)	0.4
90 ° forward flexion	-17.0 (8.0)	-11.0 (6.0)	-14.0 (8.0)	-13.0 (9.0)	0.3
120° forward flexion	-23.0 (8.0)	-15.0 (7.0)	-18.0 (10.0)	-18.0 (9.0)	0.046*

[1] Mean (standard deviation)
[2] Wilcoxon rank sum exact test comparing the difference of affected – unaffected of the dominant vs nondominant sides
* Significant at $p < 0.05$ comparing the difference between affected – unaffected sides on with cancer on the dominant side and on the nondominant side.

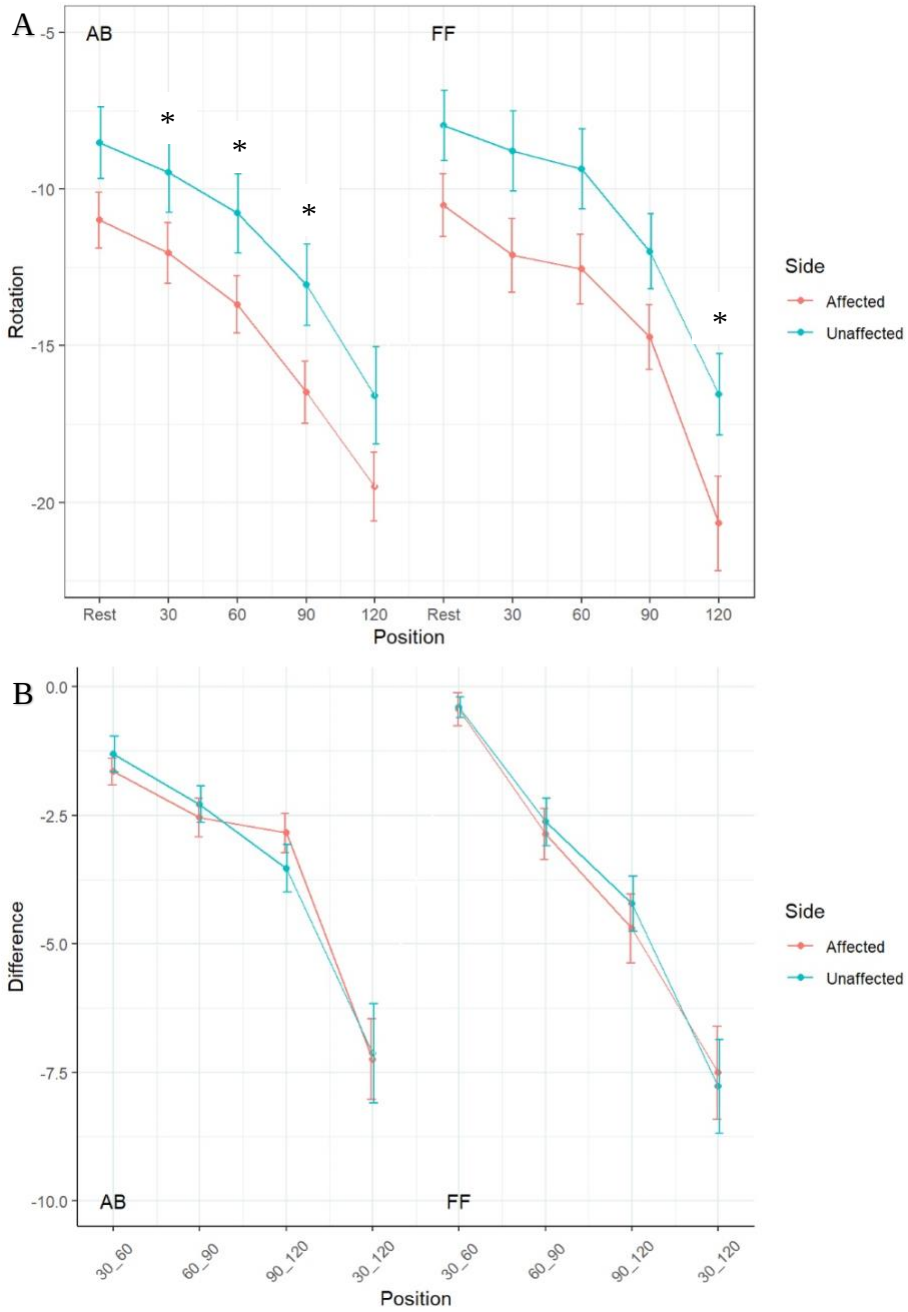

Figure 27. Sternoclavicular elevation (in degrees) during (A) abduction (AB) and forward flexion (FF) and (B) the amount of sternoclavicular elevation (in degrees) that occurred between 30° - 60°, 60° - 90°, 90° - 120°, and 30° - 120° of abduction and forward flexion. Error bars represent standard deviation.

Sternoclavicular Posterior Rotation

No statistically significant (p>0.05) mean differences (affected – unaffected sides) were noted in the amount of SC posterior rotation across HT angles. See Table 20. Those who had cancer on their dominant side moved significantly less into SC posterior rotation compared to the unaffected side than those who had cancer on their nondominant side from 90° - 120° of abduction ($p = 0.031$). See Table 21 and Figure 28. Regression analysis revealed that the addition of potential covariates did not affect SC posterior rotation (p>0.05), data not shown.

Table 20. Sternoclavicular posterior rotation of the affected side vs the unaffected side during abduction and forward flexion, expressed at specific angles and amount moved between those specific angles.

Humerothoracic Angle	**Affected**, n=26[1]	**Unaffected**, n=26[1]	***p*-value**[2]
Rest	-6 (11)	-13 (14)	0.10
30° abduction	-10 (10)	-13 (12)	0.4
60° abduction	-10 (10_	-13 (13)	0.4
90° abduction	-9 (11)	-14 (14)	0.3
120° abduction	-8 (13)	-14 (16)	0.3
30° forward flexion	-3 (12)	-7 (15)	0.4
60 ° forward flexion	-2 (12)	-8 (16)	0.2
90 ° forward flexion	1 (12)	-4 (18)	0.2
120° forward flexion	0 (24)	-4 (19)	0.3
30° - 60° abduction	0.04 (2.02)	-0.27 (1.71)	0.4
60° - 90° abduction	0.87 (2.60)	0.25 (2.80)	0.4
90° - 120° abduction	1.9 (5.2)	0.1 (4.4)	0.4
30° - 120° abduction	3 (9)	0 (7)	0.3
30° - 60° forward flexion	1.47 (2.74)	0.88 (1.77)	0.8
60° - 90° forward flexion	4.4 (4.3)	3.8 (4.0)	> 0.9
90° - 120° forward flexion	0 (7)	-1 (6)	0.8
30° - 120° forward flexion	6 (10)	4 (9)	0.2

[1]Mean (standard deviation)
[2] Wilcoxon rank sum test

Table 21. Sternoclavicular posterior rotation (in degrees) on the affected and unaffected sides expressed as a function of hand dominance.

Humerothoracic Angle	**Cancer on dominant side,** n=12[1]		**Cancer on nondominant side,** n=14[1]		***p*-value[2]**
	Affected	**Unaffected**	**Affected**	**Unaffected**	
Rest (abduction and forward flexion)	-8 (11)	-18 (13)	-4 (11)	-8 (13)	0.3
30° abduction	-11 (11)	-19 (15)	-3 (17)	-9 (16)	0.5
60° abduction	-11 (12)	-20 (16)	-2 (18)	-9 (17)	0.4
90° abduction*	-11 (12)	-20 (17)	0 (20)	-9 (16)	0.5
120° abduction*	-12 (11)	-19 (16)	5 (25)	-10 (15)	>0.9
30° forward flexion	-6 (11)	-14 (15)	2 (16)	-3 (16)	0.7
60 ° forward flexion	-6 (10)	-12 (15)	4 (17)	-1 (20)	>0.9
90 ° forward flexion	-1 (12)	-9 (16)	8 (20)	4 (22)	0.6
120° forward flexion	-1 (15)	-10 (19)	8 (23)	1 (19)	0.6

[1] Mean (standard deviation)

[2] Wilcoxon rank sum exact test comparing the difference of affected – unaffected of the dominant vs nondominant sides

* When cancer was on the dominant side, the clavicle moved less into posterior rotation than the unaffected clavicle than when the cancer was on the nondominant side from 90° - 120° of abduction ($p = 0.031$)

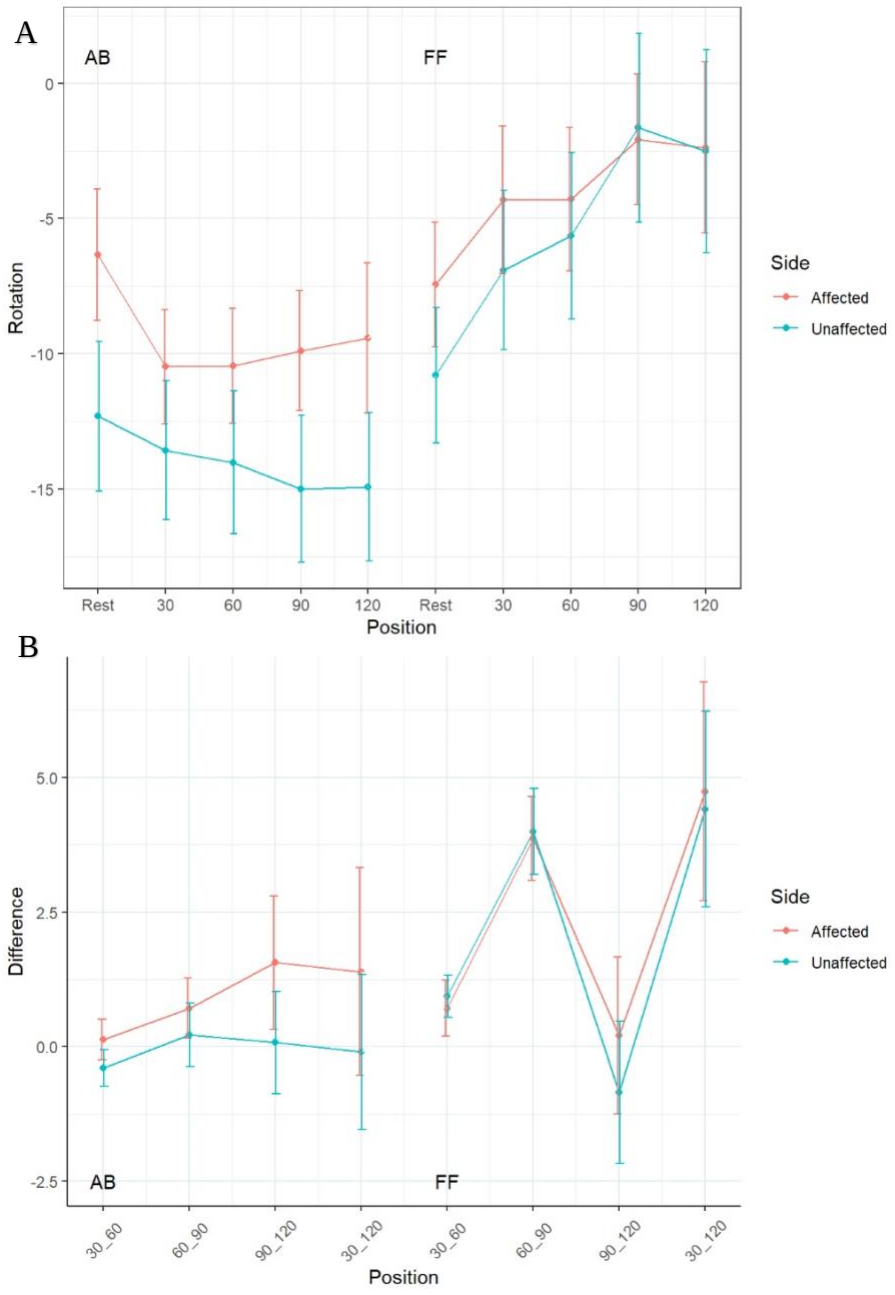

Figure 28. Sternoclavicular posterior rotation (in degrees) during (A) abduction (AB) and forward flexion (FF) and (B) the amount of sternoclavicular posterior rotation (in degrees) that occurred between 30° - 60°, 60° - 90°, 90° - 120°, and 30° - 120° of abduction and forward flexion. Error bars represent standard deviation.

Aim 3 (Relationships between 1) PSS and radiation dose and 2) PSS and EI

Penn Shoulder Score results of the breast cancer survivors are shown below. All 26 breast cancer survivors completed the PSS. On average, when the entire group was analyzed together, the mean total PSS score was high (91.8 / 100), pain was low (2.2 / 30), and satisfaction was high (8.5 / 10). See Table 22.

Table 22. Penn Shoulder Score expressed as mean +/- standard deviation (range)

Total Score	Function Subscore (# / 60)	Satisfaction Subscore (# / 10)	Pain Subscore[1] (#/30)	Pain at Rest (#/10)	Pain with normal activity (#/10)	Pain with strenuous activity
91.8 +/- 11.3 (65 – 100)	55.4 +/- 6.0 (39 – 60)	8.5 +/- 2.5 (2 – 10)	27.8 +/- 3.5 (19 – 30)	0.2 +/- 0.6 (0 – 2)	0.5 +/- 1.1 (0-4)	1.4 +/- 2.3 (0.6)

[1] Pain subscscore is the sum of 3 pain question scores.

When the individual questions about specific shoulder functional activities were examined, certain functional movements were reported to be more difficult than others. See Table 23. For example, 12 of the 26 participants (almost 50% of the participants) had some difficulty placing a 1-gallon container on an overhead shelf without bending their elbows, with 3 (11.5%) reporting much difficulty and one participant (3.8%) reporting that they could not do that activity at all. Seven participants (26.9%) reporting having 'some difficulty' sleeping on their affected side and / or carrying a bag of groceries with their affected arm. Nine (34.6%) participants reported some difficulty 'throwing overhand/swim/overhead racquet sports' and almost 1/5 reported some difficulty returning to their normal sport or hobby with 2 participants (7.7%) not able to return to that sport/hobby at all. Conversely, some activities of daily living were reported as 'no difficulty' by 100% of the breast cancer survivors (toileting and combing their hair).

Radiation treatment parameters varied among participants. These plans are complex and consist of multiple variables including but not limited to total dose, whole breast dose, boost dose, fraction dose of the whole breast, boost fraction dose, number of fractions for the whole breast, and number of fractions for the boost as applicable. Although the average dose of radiation in the group as a whole was 5083 cGy, with total breast dose 4298 cGy and 1078cGy lumpectomy boost dose, this does not accurately reflect the variety of treatment plans the participants received.

Table 23. Penn Shoulder Score results. Data expressed as n (%).

	No difficulty	Some difficulty	Much Difficulty	Can't do at all	Did not do before injury
Reach the small of your back to tuck in your shirt with your hand	23 (88.5%)	1 (3.8%)	1 (3.8%)	1 (3.8%)	0 (0%)
Wash the middle of your back / hook bra	17 (65.4%)	7 (26.9%)	0 (0%)	2 (7.7%)	0 (0%)
Perform necessary toileting activities	26 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Wash the back of opposite shoulder	19 (73.1%)	4 (15.4%)	3 (11.5%)	0 (0%)	0 (0%)
Comb hair	26 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Place hand behind head with elbow held straight out to the side	22 (84.6%)	3 (11.5%)	1 (3.8%)	0 (0%)	0 (0%)
Dress self (including put on coat and pull shirt off overhead)	22 (84.6%)	4 (15.4%)	0 (0%)	0 (0%)	0 (0%)
Sleep on affected side	19 (73.1%)	7 (26.9%)	0 (0%)	0 (0%)	0 (0%)
Open door with affected arm	24 (92.3%)	2 (7.7%)	0 (0%)	0 (0%)	0 (0%)
Carry a bag of groceries with affected arm	19 (73.1%)	7 (26.9%)	0 (0%)	0 (0%)	0 (0%)
Carry a briefcase / small suitcase with affected arm	20 (76.9%)	6 (23.1%)	0 (0%)	0 (0%)	0 (0%)
Place a soup can (1-2 lb) on a shelf at shoulder height without bending your elbow	24 (92.3%)	2 (7.7%)	0 (0%)	0 (0%)	0 (0%)
Place a one-gallon container (8-10 lb) on a shelf at shoulder level without bending your elbow	14 (53.8%)	10 (38.5%)	2 (7.7%)	0 (0%)	0 (0%)
Reach a shelf above your head without bending your elbow	23 (88.5%)	3 (11.5%)	0 (0%)	0 (0%)	0 (0%)
Place a soup can (1-2 lb) on a shelf overhead without bending your elbow	24 (92.3%)	2 (7.7%)	0 (0%)	0 (0%)	0 (0%)
Place a one-gallon container (8-10 lb) on a shelf overhead without bending your elbow	10 (38.5%)	12 (46.2%)	3 (11.5%)	1 (3.8%)	0 (0%)
Perform usual sport/hobby	19 (73.1%)	5 (19.2%)	0 (0%)	2 (7.7%)	1 (3.8%)
Perform household chores (cleaning, laundry, cooking)	23 (88.5%)	3 (11.5%)	0 (0%)	0 (0%)	0 (0%)
Throw overhand/swim/overhead racquet sports	15 57.7%)	9 (34.6%)	0 (0%)	2 (7.7%)	2 (7.7%)
Work full-time at your regular job	25 (96.2%)	0 (0%)	0 (0%)	0 (0%)	1 (3.8%)

Therefore, radiation treatment plans were further broken down in Tables 24 and 25. Three participants had regional lymph node radiation (RLNR): two participants had radiation to the supraclavicular area (S3 & S27), and 1 had radiation to both the supraclavicular and axillary regions (S30). The remainder of the participants did not receive intentional regional lymph node radiation (n=23, 88%). Twenty (77%) participants had hypofractionated RT and 5 (19%) had conventional fractionated whole breast radiation therapy, with hypofractionated radiation therapy defined as fraction radiation dose $\geq$2.0 Gy(43). Two participants had radiation from an outside provider for which were unable to obtain fractionation data.

Table 24. Radiation treatment details by patient shown as mean (standard deviation).

S#[1]	Total Dose in cGy[2]	Whole Breast dose in cGy	Lumpectomy Boost Dose in cGy	RLNR[3]	Dose per fraction to whole breast in cGy (#Fx[4])	Dose per Fraction for Boost in cGy (#Fx)	Dermatitis (Max Grade) Skin Rating / CTC AE4[5]	Total # Fract-ions	PSS total score
S2	5240	4240	1000	No	265 (16)	250 (5)	2	21	98
S3	6000	5000	1000	Yes	200 (25)	200 (5)	3	30	92
S4	5005	4005	1000	No	267 (15)	200 (5)	2	20	99
S5	4240	4240	na	No	265 (16)	na	1	16	85
S7	5240	4240	1000	No	265 (16)	250 (4)	1	20	98
S9	5240	4240	1000	No	265 (16)	250 (4)	1	20	93
S10	5240	4240	1000	No	265 (16)	200 (5)	2	21	99
S11	6040	5040	1000	No	180 (28)	200 (5)	2	33	88
S12	5490	4240	1250	No	265 (16)	250 (5)	1	21	98
S13	3600	2600	1000	No	520 (5)	250 (4)	1	9	68
S14	4240	4240	na	No	256 (16)	na	1	16	100
S15	5490	4240	1250	No	256 (16)	250 (5)	2	21	96

S16	5240	4240	1000	No	256 (16)	250 (4)	1	20	97
S18	5240	4240	1000	No	265 (16)	250 (4)	1	20	94
S19	5490	4240	1250	No	265 (16)	250 (5)	1	21	69
S20	5490	4240	1250	No	265 (16)	250 (5)	1	21	100
S21	4240	4240	na	No	265 (16)	na	1	16	77
S22	4240	4240	na	No	265 (16)	na	1	16	100
S23	5240	4240	1000	No	*	*	*	20	98
S24	6040	5040	1000	No	180 (28)	200 (5)	3	33	100
S25	4240	4240	na	No	265 (16)	na	1	16	100
S26	4240	4240	na	No	265 (16)	na	1	16	72
S27	6290	5040	1250	Yes	180 (28)	250 (5)	2	33	100
S28	5490	4240	1250	No	265 (16)	250 (5)	1	21	65
S29	5940	5040	900	No	180	*	*	33	100
S30	5240	4240	1000	Yes	265 (16)	250 (4)	1	20	97

[1]S# = participant number
[2]cGy = centigray
[3]RLNR = regional lymph node radiation (axillary and/or supraclavicular)
[4] = fractions (daily radiation dose)
[5]CTCAE4 = Common Terminology Criteria for Adverse Events skin reaction grade given at the end of radiation by the radiation oncologist
[*] Unknown (outside provider, information not available)

Multiple regression and Spearman rank correlation analyses were used to determine the relationships between the patient-reported outcome measure PSS and radiation dose (Hypothesis 3.1) and EI of the PMaj, PMin, and SA (Hypothesis 3.2)

Table 25. Radiation dose analysis by muscle group expressed as mean (standard deviation)

	V10[1]	V15	V20	V30	V40	Min	Mean	Max
PMaj	66.9 (13.3)	64.7 (13.3)	62.4 (13.3)	57.7 (17.2)	47.0 (19.5)	1.0 (0.8)	27.6 (7.7)	50.7 (7.2)
PMin	83.7 (13.5)	82.2 (14.2)	79.8 (14.9)	74.3 (20.8)	51.7 (46.5)	6.6 (13.9)	34.1 (9.0)	46.5 (8.3)
SA	37.4 (10.1)	34.8 (9.9)	32.9 (9.8)	28.5 (10.7)	21.9 (10.3)	0.4 (0.2)	15.8 (4.6)	50.4 (7.2)

[1] V10 refers to the percent of each muscle that received at least 10 Gray (Gy) of radiation. V15 refers to the percent of each muscle that received at least 15Gy of radiation, etc.

The relationship between patient-reported shoulder function (PSS) & RT (Hypothesis 3.1)

Muscle-specific radiation dose analysis was not available for 2/26 participants due to the scans being performed at an outside facility using software that was not compatible with Velocity. Total radiation dose was available for all 26 participants. Therefore, a total of 24/26 radiation simulation scans were utilized for this aim.

Linear regression

Multiple linear regression analyses were performed to determine if RT affected PSS scores. SA mean dose, SA_V30 dose, tumor quadrant location, total RT dose, PMaj mean dose, PMin mean dose, and V30 doses of the PMin and PMaj, and RLNR were used in these models. Model 1 included SA mean and V30 doses, tumor location/breast quadrant, and total RT dose. Model 2 included PMaj and PMin mean and V30 doses as well as RLNR and total RT dose. Model 3 included total RT dose, boost dose, and RLNR. All models corrected for other variables included in that particular model. Multiple models were utilized as many predictor variables were highly correlated, negating their use within the same model.

Model 1:

No statistically significant results were found in this model. SA mean dose, SA V30 dose, tumor location/quadrant, and total RT dose did not significantly affect PSS score (all *p*-values and *q*-values > 0.2). See Table 26.

Table 26. Multiple linear regression Model 1 demonstrating the effect of the serratus anterior radiation dose, tumor quadrant/location, and total radiation dose on the Penn Shoulder Score. Only trends shown. Nonsignificant findings (p>0.05, q>0.05) are not shown.

	Penn shoulder score, total score		Penn shoulder score, function subscore		Penn shoulder score, pain subscore	
	β (95%CI)	*p*-value	β (95%CI)	*p*-value	β (95%CI)	*p*-value
Serratus anterior mean dose (cGy) [1]	-1.1[2] (-9.8,7.5)	0.8	-0.46 (-4.8, 3.9)	0.8	-0.53 (-3.1, 2.1)	0.9
Serratus anterior V30[2] (%)	0.94 (-2.6, 4.5)	0.6	0.35 (-1.5, 2.2)	0.7	0.38 (-0.7, 1.5)	0.5
Tumor Quadrant[3]	-2.1 (-18, 14)	0.8	0.53 (-8.9, 7.8)	0.9	-1.2 (-6.2, 3.7)	0.6
Total Breast + Lumpectomy Boost Dose	0.00 (-0.01, 0.02)	0.6	0.00 (-0.01, 0.01)	0.8	0.00 (0.00, 0.01)	0.12

[1]cGy = centigray, [2] data shown as βeta (95% confidence internal)
[2] V30 = percent of muscle that received at least 30 Gy of radiation
[3] Quadrant = upper, lower, unknown, axillary tail, overlapping quadrants, or central
*All q-values (adjustment for multiple testing) = 0.2

Model 2:

Trends were noted for the PMin mean radiation dose to negatively affect the overall PSS total score and PSS function subscores, but these were not significant after correction for multiple testing. See Table 27. As PMin V30 dose increased, so did the PSS total, PSS function, and PSS pain scores although these were also not statistically significant after correction for multiple testing. Higher total RT dose increased PSS pain but this was also not statistically significant after correction for multiple testing (p=0.049, q=0.2).

Model 3:

A third multiple linear regression model was utilized to determine the effect of total RT dose, boost dose, and RLNR on PSS scores. Total RT dose increased total PSS score as well as PSS function, pain, and satisfaction subscales (p=0.015 - 0.032) but these were not significant after correction for multiple testing (q>0.05). See Table 28. Boost dose and RLNR did not have a significant effect on PSS scores in this model (p and q >0.05).

Table 27. Multiple linear regression Model 2 demonstrating the effect of pectoralis minor radiation dose on the Penn Shoulder Score. Only trends shown. Nonsignificant findings (p>0.05, q>0.05) are not shown.

	Penn shoulder score, total score		Penn shoulder score, function subscore		Penn shoulder score, pain subscore	
	β (95%CI)	*p*-value	β (95%CI)	*p*-value	β (95%CI)	*p*-value
Pectoralis minor mean dose (cGy) [1]	-4.1[2] (-7.8,-0.48)	.03	-2.3 (-4.0, -0.54)	.015	-1.2 (-2.3, 0.00)	.05
Pectoralis minor V30 (%)	1.8 (0.24, 3.4)	.027	0.94 (0.20, 1.7)	.17	0.53 (0.03, 1.0)	.038

[1]cGy = centigray, [2] data shown as βeta (95% confidence internal)
*All q-values (adjustment for multiple testing) = 0.2

Table 28. Multiple linear regression Model 3 showing trend for total radiation dose to affect Penn Shoulder Score. Only trends shown. Nonsignificant findings (p>0.05, q>0.05) are not shown.

	Penn shoulder score, total score		Penn shoulder score, function subscore		Penn shoulder score, satisfaction subscore	
	β (95%CI)	*p*-value	β (95%CI)	*p*-value	β (95%CI)	*p*-value
Total radiation dose (cGy)[1]	0.01 (0.00,0.03)	.03	-.01 (0.00, 0.02)	.02	0.00 (0.00, 0.01)	.02

Pectoralis minor V30 (%)	1.8 (0.24, 3.4)	.027	0.94 (0.20, 1.7)	.17	0.53 (0.03, 1.0)	.038

[1]cGy = centigray, [2] data shown as βeta (95% confidence internal)
*All *q*-values (adjustment for multiple testing) = 0.2

Correlation Analyses

Spearman rank correlations coefficients were performed to determine the general relationship between the PSS and estimated absorbed radiation dose to the muscles of interest. One statistically significant correlation was found between PMaj V20 dose and patient reported satisfaction, with a Spearman's rank correlation coefficient of 0.42 ($p<0.05$), reflecting a fair relationship between the 2 variables. (Portney&Watkins). See Table 29.

Table 29. Spearman rank correlation coefficient (r_s) demonstrating the correlation between Penn Shoulder Score and V10-V40 doses[1] as well as estimated absorbed pectoralis major radiation dose in Gray (Gy). Data presented as r_s.

Penn Shoulder Score	V10[1]	V15[1]	V20[1]	V30[1]	V40[1,2]	Min-imum Dose	Mean Dose	Max-imum Dose
Total	0.23	0.19	0.26	0.14	-0.27	0.20	0.03	-0.04
Function	0.18	0.14	0.20	0.04	-0.26	0.23	-0.04	-0.02
Pain	0.28	0.28	0.25	0.21	-0.06	0.17	0.07	0.03
Satisfaction	0.32	0.30	0.42*	0.32	-0.06	0.18	0.22	-0.03

[1]V10 = percent of muscle receiving at least 10Gy, V15 = percent of muscle receiving at least 15Gy, V20 = percent of muscle receiving at least 20Gy, V30 = percent of muscle receiving at least 30Gy, V40 = percent of muscle receiving at least 40Gy.
[2] n=24 for each group except V40, which only had 23 as 1 participant had total radiation dose less than 4000Gray.
*Significant at $p<0.05$

Many statistically significant positive correlations ($p<0.05$) were found between the PMin estimated absorbed radiation dose and PSS pain and satisfaction subscales. See Table 29. All noted correlations were between the V-doses and PSS subscales, not with the minimum, mean, or maximum dose and PSS. There was a moderate to good positive relationship between PMin V30

dose and patient reported satisfaction (r_s = 0.522, p<0.05), and fair positive relationships between V10-V20 doses and both pain and satisfaction (r_s = 0.408 – 0.47, p<0.05).

A few weak positive relationships were found using Spearman rank correlation analysis between PSS pain score and PMin V15dose, V40 dose, and mean dose (r_s= 0.39 -0.45, p<0.05). See Table 30. The SA V15, V40 and mean dose also had weak positive correlations with PSS pain score (r_s= 0.39 -0.44, p<0.05). See Table 31.

Table 30. Spearman rank correlation coefficient (r_s) demonstrating the correlation between Penn Shoulder Score and V10-V40 doses[1] as well as estimated absorbed pectoralis minor radiation dose in Gray (Gy). Data presented as r_s.

Penn Shoulder Score	V10[1]	V15[1]	V20[1]	V30[1]	V40[1,2]	Min-imum Dose	Mean Dose	Max-imum Dose
Total	0.31	0.30	0.32	0.23	0.07	0.03	0.22	0.16
Function	0.30	0.29	0.32	0.21	0.10	-0.00	0.23	0.20
Pain	0.43*	0.43*	0.47*	0.42*	0.23	-0.06	0.34	0.24
Satisfaction	0.41*	0.41*	0.42*	0.52*	-0.07	0.15	0.34	0.28

[1]V10 = percent of muscle receiving at least 10Gy, V15 = percent of muscle receiving at least 15Gy, V20 = percent of muscle receiving at least 20Gy, V30 = percent of muscle receiving at least 30Gy, V40 = percent of muscle receiving at least 40Gy.
[2] n=24 for each group except V40, which only had 23 as 1 participant had total radiation dose less than 4000Gray.
*Significant at p<0.05

Table 31. Spearman rank correlation coefficient (r_s) demonstrating the correlation between Penn Shoulder Score and V10-V40 doses[1] as well as estimated absorbed serratus anterior radiation dose in Gray (Gy). Data presented as r_s.

Penn Shoulder Score	V10[1]	V15[1]	V20[1]	V30[1]	V40[1,2]	Min-imum Dose	Mean Dose	Max-imum Dose
Total	0.04	0.15	0.06	0.04	0.11	0.17	0.20	0.08
Function	0.05	0.16	0.07	0.07	0.13	0.22	0.24	-0.03
Pain	0.32	0.41*	0.33	0.31	0.39*	0.16	0.44*	0.00

Satisfaction	0.25	0.32	0.26	0.33	0.27	0.34	0.45*	-0.04

[1]V10 = percent of muscle receiving at least 10Gy, V15 = percent of muscle receiving at least 15Gy, V20 = percent of muscle receiving at least 20Gy, V30 = percent of muscle receiving at least 30Gy, V40 = percent of muscle receiving at least 40Gy.
[2] n=24 for each group except V40, which only had 23 as 1 participant had total radiation dose less than 4000Gray.
*Significant at $p<0.05$

PSS & EI (Hypothesis 3.2)

No statistically significant relationships were found between the PSS and PMaj, PMin, and SA EI. Regression analysis demonstrated no statistically significant relationships between the PSS and PMaj, PMin, and SA EI scores, with all *p*-values $\geq$0.12 and all *q*-values $\geq$0.4. Spearman correlations were also all statistically nonsignificant, and ranged from -0.025 to 0.328, with all 2-tailed p-values > 0.111. Data not shown.

Exploratory Analyses:

Multiple linear regression analysis models were also used to determine the effect of RT treatment plan (RLNR, total dose, boost dose, tumor location/breast quadrant) on radiation dose to the 3 muscles of interest. The first model used tumor location/breast quadrant alone, the second included RLNR alone, and the third included total RT dose, boost dose, and RLNR. All models corrected for other variables included in that model.

Effect of RLNR on estimated absorbed radiation dose to the PMaj, PMin, and SA (without adjusting for other factors)

Multiple linear regression was used to evaluate the relationship between RLNR and radiation dose to the 3 muscles of interest. Statistically significant differences were found between those who had RLNR and those who did not, without adjusting for other factors. On average, those who had RLNR had 9.6Gy higher mean dose to the PMaj (β=9.6, 95% CI=(2.6, 17), p = 0.011, q = 0.033) even after correction for multiple testing. Additionally, those who had RLNR had, on average, 24% more of the PMaj exposed to 30Gy or more than those who did not have RLNR, even after correction for multiple testing (β=24, 95%CI = (12,37), p = 0.001, q = 0.006). See Table 31. Trends were also noted for those who had RLNR having a greater percent of the PMin and SA exposed to at least

30Gy (V30) (p = 0.049 and 0.054 respectively) but this was not significant after correction for multiple testing (q = 0.081 for both).

Effect of RT treatment plan on estimated absorbed radiation dose to the PMaj, PMin, and SA

Multiple linear regression was used to determine the effect of radiation treatment (boost dose, RLNR treatment, and total RT dose) on estimated absorbed radiation dose to the muscles of interest. As radiation boost dose increased, the mean dose to the PMaj also significantly increased after adjusting for other factors in the model (β=0.05, 95% CI = 0.04, 0.07), p<0.001, q<0.001). As boost dose increased, the percent of the PMaj exposed to at least 30Gy of radiation (V30) also increased, (β=0.09, 95% CI (0.06, 0.13), p<0.001, q<0.001).

RLNR significantly increased the PMaj and PMin mean doses as well as the PMaj and PMin V30 doses, even after correction for multiple testing. See Table 32. Conversely, as total RT dose increased, the mean dose to the PMaj slightly decreased (β=-0.02,95% CI (-0.03, -0.01), p=0.006, q=0.015) and the PMaj V30 dose also slightly decreased (β=-0.04, 95% CI (-0.07, -0.02), p=0.005, q=0.015). The estimated absorbed mean RT dose to the SA increased with an increase in total RT dose (β=0.03, 95% CI (0.00, 0.05), p = 0.006, q = 0.015). As boost dose increased, there was also a trend for the PMin V30 dose to also increase but this was not statistically significant after correction for multiple testing (β=0.10, 95% CI (0.00, 0.20), p=0.048, q=0.089).

Table 32. Multiple linear regression results demonstrating the significant effect of regional lymph node radiation on estimated absorbed radiation dose to the pectoralis major and minor. Serratus anterior doses were not significant predictors in the model and are not shown (p>0.05).

	Beta (β)	95% confidence interval	p-value	q-value[4]
PMaj[1] mean dose	13	10, 16	<0.001*	<0.001*
PMin[2] mean dose	14	3.2, 24	0.016*	0.036*
PMaj V30 dose[3]	31	25, 37	<0.001*	<0.001*
PMin V30 dose[3]	36	18, 54	0.002*	0.007*

* Statistically significant at <0.05; [1]PMaj = pectoralis major, [2]PMin = pectoralis minor
[3]V30 dose = percent of muscle exposed to at last 30Gray of radiation; [4]False discovery rate correction for multiple testing

Effect of tumor location (breast quadrant) on muscle-specific radiation dose

Those who had tumors in the lower quadrant of the breast (inner and outer lower quadrants combined) had significantly higher SA mean dose and SA V30 values than those who had tumors in the upper quadrant (inner and outer upper quadrants combined): β=3.5, 95% CI (1.0, 5.9), p=0.008, q=0.025 and SA V30 value: β=8.7, 95% CI = (3.0, 14), p=0.006, q=0.025). There were no statistically significant relationships between tumor quadrant and PMaj or PMin mean and V30 values.

Relationship among mean estimated absorbed dose to each muscle of interest

Additional Spearman rank correlation analyses were run to determine the relationship between the mean, minimum, and maximum doses to each muscle. Good to excellent relationships were found between the PMaj mean and PMin mean (r_s = 0.81). The mean dose of the PMaj and PMin were not significantly related to SA mean dose (r_s = 0.012 and 0.40, respectively).

CHAPTER 6: DISCUSSION

The purpose of this study was to better understand the relationships among 3D shoulder kinematics, skeletal muscle morphology, patient reported shoulder outcomes, and post-lumpectomy radiation therapy in breast cancer survivors at least 1 year after the completion of adjuvant RT. In summary, we found that the clavicle on the affected side is elevated during shoulder elevation compared to the nonaffected side, although clinical significance of this finding is unclear. Patient reported outcomes reported as PSS scores are high on average although many participants had significant difficulty with certain ADLs and complained of stiffness and / or pain in not just their shoulder, but also their chest wall, arm, and elbow. We validated the use of B-mode ultrasound in healthy participants for PMaj and PMin CSA and EI values, but not for the SA. B-mode US was not able to detect differences in CSA or EI between the affected or unaffected sides in breast cancer survivors. Linear regression revealed possible relationships between PSS scores and PMin as well as total RT doses, and correlation analysis demonstrated statistically significant relationships between PSS scores and PMin, PMaj, and SA radiation doses that warrant further investigation.

Specific Aim 4 (Ultrasound reliability study in healthy participants)

The primary finding of our US reliability study was that B-mode musculoskeletal ultrasound can reliably be used by 1 rater with moderate training using the described methodology to obtain PMaj

and PMin CSA and EI data in a healthy cohort of middle-aged women. Therefore, we used these methodologies with our breast cancer cohort analyses. The SA CSA and EI data had poor reliability, and the data was used in the breast cancer cohort in an exploratory nature only. Therefore, our Aim 4 hypotheses were partially met.

Ultrasound CSA and EI were chosen as potential biomarkers of skeletal muscle health within the radiation field following lumpectomy and radiation based on previous literature that supports the use of US to examine skeletal muscle morphology(137,207,217,218) and the ease, availability, low cost, and non-invasiveness of B-mode US. In retrospect however, much of the previous literature used panoramic mode US to capture musculoskeletal CSA(50,130,131). Panoramic US was not performed as part of this study due to the complexity of scanning and lack of training of RB who performed the ultrasound scans, and lack of finances and time to support the hiring of outside personnel to perform this more complicated technique. Another limitation is the lack of validation against a gold standard (130); ideally the CSA and EI of US images would be compared to a gold standard such as MRI. However, MRI was not utilized in this study due to financial limitations and time constraints.

To the best of our knowledge, no previous research has been performed to validate reliability of non-panoramic musculoskeletal B-mode US to determine CSA and EI of the serratus anterior or pectoralis minor muscles. During self-directed ultrasound training, RB consulted with radiologists, anesthesiologists, and plastic surgeons to develop the US methodology as PMin and SA are not commonly scanned. In fact, when RB contacted Sonosite, the ultrasound manufacturer, they were unable to help with imaging of any of the muscles of interest. This prompted consultation with the experts indicated, and adaptation of current anesthesiology techniques (PECSI and PECSII blocks) to image the PMaj, PMin, and SA. Some literature does exist to guide US of the PMaj as that is a more commonly injured muscle (219). Regarding EI, our research was supported by previous validation of EI grayscale analysis using ImageJ (134).

The SA was more difficult to scan as it required counting the ribs as the ultrasound transducer was moved inferiorly and laterally across the ribcage. It is possible that a more experienced technician would have more reliable results (130,219). It is also possible that variation in breath stage during image acquisition affected reliability as rib expansion with breath has the potential to affect SA ROI due to the SA attachment on the ribs. Every effort was taken to take images at the end of a

normal, relaxed exhale but this may have added variability that was reflected in the non-reliable SA measures.

Our findings suggest that B-mode MSKUS with ImageJ analysis can reliability be used to determine EI and CSA of the PMaj and PMin, but not the SA, in a healthy adult female population. Therefore, these measurements were utilized in the breast cancer survivor cohort. However, B-mode MSKUS with Image J analysis for SA data was not reliable, and analysis of the SA data was performed in an exploratory nature only in the breast cancer survivor cohort.

Specific Aim 2: Breast cancer survivor skeletal muscle ultrasonography

We did not demonstrate a significant difference in CSA or EI when examining the main group effect of affected versus unaffected sides of the PMaj, PMin, or SA as stated in Hypotheses 2.1 and 2.2. This could be because the study was underpowered to detect changes in EI and CSA, the variability was too high, B-mode ultrasound was not sensitive enough to detect these changes as performed, or because actual differences did not exist.

Our study was powered to detect differences in ST upward rotation, not EI or CSA. A secondary unofficial power analysis was performed based on Yang et al (51) who found significant differences in vaginal wall EI following surgery + RT as compared to a control group who had surgery without RT when combined with advanced imaging methods in women undergoing treatment for endometrial cancer. That power analysis calculation suggested an 'n' of 18 (9 in each group), which was significantly lower than our number of participants and had led us to believe that we would capture significant effects. A post-hoc power analysis was run based on 2-tailed test using Wilcoxon signed rank test which was used in the study statistics, with an effect size of 0.5 and alpha of 0.05. Power was determined to be 0.6664, supporting the hypothesis that this study was underpowered to detect differences in EI or CSA. In order for the power to reach 95%, the study sample should have been 57, more than double what was used in this study.

Another potential reason that we did not find significant differences in EI or CSA of any muscles between the affected and unaffected sides is that the variability in the data was too high. For example, the difference between the mean EI of the affected and unaffected PMaj was 3.93, but the standard deviation was greater than 16 for both the affected and unaffected sides. Similar trends existed for the PMin and SA.

It is also possible that B-mode musculoskeletal ultrasound was not sensitive enough to detect small differences in EI that existed between groups, or that US did not accurately reflect changes in fibrosis. Previous research is conflicted on whether EI reflects fibrous tissue (51,121) or fatty infiltration (133), or both (137). It is possible that other, more expensive methods of soft tissue analysis such as MRI or CT could detect these subtle differences in tissue morphology. Tissue biopsies would be another way to validate the use of US to determine EI and has been performed in dogs (121), but this did not meet ethical or financial constraints of our study.

Additionally, it is possible that differences did not exist between the affected and unaffected side in terms of EI of the 3 muscles of interest. Based on 20 years of clinical experience during which RB and other clinicians have palpated differences in tissue texture between radiated and nonradiated sides we expected to find differences in tissue EI. However, it is possible that these differences represent changes in tissue stiffness as demonstrated by Lipps et al (74), and not differences in EI.

Variability may be minimized in the future by using more standardized methodology including practicing normal breathing techniques prior to image acquisition, especially as it has the potential to affect SA CSA due to changes in rib position with respiration. Additionally, more specific marking of the scan site as per Pareja-Blanco et al. who used a transparent acetate sheet to accurately reproduce their image acquisition site, could improve reliability (209). RAB marked locations outside of, but not directly on, the ROI due to the tendency of the US gel to smear the marker. Future research should consider using a permanent marker that would not smear with the US gel, or an acetate sheet, to more precisely locate the ROI and improve location identification and reliability.

Our findings may have been different than those of Yang et al (51) for several reasons. It is possible that the type of radiation used in the endometrial cancer population (brachytherapy and/or external beam radiation) affects tissues differently than external beam radiation used in breast cancer treatments. It is also possible that vaginal wall tissue is more sensitive to radiation effects than chest wall musculature. Additionally, the advanced US imaging techniques used by Yang et al may have been more reliable and accurate as scans were performed by a radiation oncologist and verified by both a radiologist and medical physicist as compared to RAB who performed extensive practice

and consulted with experts but who lacked formal training. For example, EI may be influenced by many factors such as the angle of the transducer relative to muscle fiber orientation, image orientation (transverse or longitudinal), region of interest / analysis selection, and compressive force of the transducer on the underlying tissues (137,220).

Wolfram et al. found significant changes in PMaj stiffness (using US shear-wave elastography) and thickness (using B-mode US) following tangential field radiation to the breast with boost following lumpectomy and SLNB (42). In their research, the sternocostal region of the PMaj had higher mean radiation dose than the clavicular portion. We chose to measure the clavicular portion in our research to avoid scanning over the inferior breast tissue on the chest wall, as this could have affected the underlying muscle image quality. Chest wall imaging location could be one reason why we did not find significant differences in EI or CSA in the PMaj and PMin muscles, as the clavicular PMaj where we took our US measures may have received less radiation than the sternocostal region based on radiation field design. However, it is important to note that other than specifying 'clavicular' or 'sternocostal' regions of the PMaj, neither Wolfram nor Lipps specified exact landmarks where their images were captured, limiting reproducibility.

Regression analysis to determine the effect of potential covariates on EI and/or CSA revealed a trend for those who had estrogen therapy to have greater CSA of the PMaj and PMin, although the trend was not statistically significant. We used estrogen as a potential covariate in our exploratory analysis as we expected that those who had estrogen-blocking medications would have decreased muscle CSA and increased muscle EI. Estrogen has a protective effect on muscle following injury as well as on muscle mass and strength through its effect on oxidative stress and skeletal muscle stem cell numbers (175–177). In fact, Rong et al proposed that estrogens protect against radiation damage and described "the impact of estrogen and ER signaling on cell cycle progression is a critical factor for their contribution to radioresistance" (174). Additionally, RT may prevent muscle hypertrophy in rats undergoing resistance training due to their adverse effect on stem cells (Adams2002).

Based on these ideas, we had thought that it was possible that anti-estrogen therapy and radiation both concurrently inhibit muscle repair. Rong et al suggested that lung fibrosis was more common in those who had concurrent anti-estrogen and radiation treatment (174). Our study did not support this idea however, which could be due to several factors: the study being underpowered, high data

variability, not controlling the length of time hormone blockers or dose given to patients, or the lack of this proposed relationship as the q-value adjusted for multiple comparisons was not significant. Future research could examine this relationship further by improving study design to specifically examine the effect of estrogen-deprivation therapy on skeletal muscle mass, muscle strength, and function in breast cancer survivors by designing a study powered to detect such differences in breast cancer survivors with and without estrogen blocking therapies.

Our ability to directly compare our PMin and SA EI and CSA results to previous literature is limited. Shamley et al demonstrated decreased CSA of the PMin using MRI in a group of breast cancer survivors with unilateral breast cancer, but their participants had a wide variety of surgery and radiation types in contrast to our study which only included lumpectomy + SLNB. It is possible that the more aggressive (mastectomy, ALND, RLNR) treatment caused their findings to be significant, or that MRI was more sensitive to post-treatment changes than B-mode US.(112)

To the best of our knowledge, PMin EI and SA EI and CSA have not been studied in the breast cancer population in terms of skeletal muscle morphology. We included these muscles in our analysis secondary to their anatomic location on the chest wall within the radiation field predisposing them to postsurgical and postradiation changes that could affect shoulder function. Interestingly, Harrington et al(2020) did validate the use of PMin length testing using a handheld palpation meter and a motion capture system.(63) They found significant correlations between the 2 measures ($r = 00.81 – 0.87$, $p < .0.001$). The handheld palpation meter is an inexpensive and readily available tool that could be used to measure PMin length but would not add skeletal muscle morphology information as we had intended in our research.

Although the effect of adjuvant RT on skeletal muscles is not well understood, previous research has validated the effect of radiation on skin thickness. Liu et al (2010) who found an increase in skin thickness a median of 22 months post RT (125). It is possible that skin is more sensitive to radiation than skeletal muscle or was exposed to higher levels of radiation. Liu et al also used a 12-MHz linear probe which is better for superficial tissues such as skin vs the L19-5 (MHz) transducer that was used in our study. Liu et al also examined radio-frequency signals which may have contributed to the strength and significance of their findings.

The lack of significant differences of the EI and CSA variables in our study can also be explained by differences in physical activity and/or exercise training between groups that were not controlled and/or adjusted for in the statistical analyses. As deconditioning, and conversely exercise training, can influence muscle size and echo intensity (221), this could also explain the lack of treatment effect observed in our study.

The relationship of CSA to EI

Correlation analysis of CSA comparing affected versus unaffected sides demonstrates significant positive correlations between the affected PMaj and affected PMin suggesting that radiation may have a similar effect on CSA in these 2 muscle groups. This is not surprising as these two muscles are in similar anatomic locations, with the majority of the PMin muscle belly situated just deep to that of the PMaj. However, as significant positive correlations were also found between, for example, the affected serratus CSA and unaffected SA CSA, the correlation results may be due to Type I error due to a large number of analyses.

Similarly, although correlation analysis within the EI data demonstrates many significant correlations, the strongest between the affected PMaj EI and affected SA EI, it also revealed a significant relationship between the affected PMaj EI and unaffected SA EI. Again, to the best of this author's knowledge, the latter suggests more of a random effect and possibly more shared variance than clinical significance.

Therefore, due to the number of contrasting results, further research is needed to determine if CSA is significantly correlated with EI. Although surgery and radiation both may affect CSA and / or EI as shown by our data, EI reflective of fibrosis or fatty infiltration may not be the same mechanism by which CSA is affected. A muscle may have a higher fibrosis content (higher EI) and not necessarily be smaller (which may be more to disuse or random effects). In summary, we did not conclude any significant findings from the correlation analyses utilizing CSA and EI as factors.

Future research should be conducted with a longitudinal design, examining pre-RT compared to post-RT measures over 1-2 years to collect acute and chronic changes that can occur following surgery and radiation for breast cancer. This study design was not feasible due to the short timeline required for this PhD dissertation. Additionally, in future research, skeletal muscle morphology and stiffness could be collected by trained radiologists using MRI, specifically analyzing the

sternocostal PMaj in place of the clavicular PMaj (42), using an inexpensive, clinically available handheld myotonometer (222) or shear wave elastography (40,42).

Overall, ultrasound is a viable option to monitor the effect of surgery and/or RT due to the ease of use, accessibility, non-invasiveness of the procedure, and relative inexpensiveness as compared to MRI and other imaging options. With more research and technique validation, it is possible that US can provide easily attainable and non-invasive biomarkers indicative of morphological tissues changes that predispose patients to shoulder disability secondary to changes stemming from cancer treatment.

Specific Aim 1: Shoulder kinematics in breast cancer survivors

The only kinematic variable that showed a significant difference between the affected and unaffected sides was SC elevation. Sternoclavicular elevation was greater on the affected than on the unaffected sides at many angles in both forward flexion and abduction, although the amount moved between different angles (i.e. 30°-120°) was not different by treatment. This is because throughout arm elevation, regardless of plane, the clavicle started and ended more elevated on the affected versus the unaffected side.

Further adjustment for hand dominance revealed that when cancer was on the nondominant side, there was not a statistically significant difference between the affected and unaffected sides. In contrast, when cancer was on the dominant side, the affected side moved significantly more into SC elevation than the unaffected side at many angles of elevation. This data suggests that the differences noted for SC elevation are reflective of the combination of hand dominance and treatment, and not treatment alone.

Clinical relevance of these findings needs to be studied further, but the increased SC elevation may reflect protective posturing that is common in breast cancer survivors, with the shoulder more elevated on the dominant affected side (clinical experience). It may also reflect ‘shrugging’ which is an increased use of the upper trapezius that excessively elevates the shoulder as a whole during arm elevation and is often found in those with scapula dysfunction (223).

To the best of our knowledge, this is the first time that SC rotations (clinically referred to as clavicle elevation, retraction, or posterior rotation) have been directly studied in breast cancer survivors.

One study reported clavicle movement, but it was indirectly measured using landmarks on the sternum and scapula (103) rather than being measured directly on the clavicle itself (92). Most other existing kinematic literature re: breast cancer survivors has focused on ST and GH kinematics during upper extremity movement (102,103,113,224,225).

From existing research, we know that otherwise healthy people with shoulder pain exhibit 5° less SC elevation at 30° of arm elevation but then move more into SC elevation from 60° - 90° and from 90° - 120° of arm elevation compared to healthy people without shoulder pain (47). Our findings were different possibly because our factor of interest was adjuvant RT, not pain. The shoulder shrug is a common sign of weakness and decreased ROM (226). It is likely that in our study, the affected side moved more into SC elevation than the unaffected side for either of these reasons, neither of which were tested in this study, but which have previously been shown to be present in breast cancer survivors (64,191,227).

We had hypothesized that scapula upward rotation and posterior tilt would be decreased (Hypotheses 1.1 and 1.2) on the affected vs the unaffected side within the same individual. Our research did not support either of these hypotheses. In fact, ST upward rotation, ST internal rotation, SC posterior rotation, and SC retraction were not significantly different on the affected as compared to the unaffected upper extremities as a function of adjuvant RT.

We had expected to see significant differences in kinematics between the affected and unaffected sides due to the adverse sequelae of surgery and RT on anterior and anterolateral chest wall tissues (3,32). These tissues are inadvertently targeted by RT due to their anatomical location on the chest wall within the radiation field. The PMaj has been shown to be smaller and stiffer following treatment and/or compared to the unaffected side(41,74,228). In theory, such changes in PMaj morphology including increased passive resistance have the potential to decrease SC elevation, posterior rotation, or retraction via the muscles' direct attachment to the clavicle. If breast cancer survivors are not able to overcome an increased stiffness of the PMaj due to disuse atrophy or deconditioning of the agonist muscles, then the decreased SC mobility has the potential to affect ST upward rotation per the coupling mechanism(97).

We had anticipated similar changes in the PMin due to its anatomic location just deep to the superior-lateral portion of the PMaj: tightness, stiffness, shortness, or increased scar tissue. These morphological changes in the PMin could increase ST internal rotation and/or ST anterior tipping due to its attachment on the coracoid process as suggested by Borstad and Szucs who found increased ST internal rotation following breast cancer surgery (102). In that study, hand dominance, surgery type, and breast reconstruction type were not included in the analyses, limiting direct comparison to our study which controlled for these factors.

Regarding the SA, we had anticipated decreased ST upward rotation due to adverse effects of adjuvant RT on SA function as a primary mover of the scapular into upward rotation (93) . However, Brookham et al demonstrated increased effort of the SA during daily tasks despite normal SA muscle strength suggesting that it is compensating for other tissues that are either not contracting normally, or its increased effort reflects the need to overcome passive resistance of tissues (such as a stiffer PMaj) (227). It is possible that one reason we did not detect more differences in kinematic patterns in our study is that participants unintentionally increased specific muscle activities to achieve normal shoulder kinematic movement patterns as patients attempted to return to normal function after treatment.

Some trends were noted for those who took endocrine therapy and those who had RLNR, although adjustment for multiplicity of testing made these changes nonsignificant. These nonsignificant trends included more SC elevation and less ST internal rotation at specific angles of arm elevation. Additionally, those who had RLNR tended to have increased ST posterior tilt in forward flexion and decreased SC retraction in abduction at certain angles. However, these patterns were not consistent or statistically significant and need to be validated in future studies.

Very little previous research has specifically examined 3D shoulder kinematics in breast cancer survivors after lumpectomy + RT comparing the affected vs the unaffected side, limiting direct comparison to previous literature. One very recent study reviewed existing post-mastectomy shoulder kinematics and concluded that more research is needed that can be easily understood by clinicians, as the variety of kinematic analyses, methodologies, surgical and rehabilitation conditions, etc. make it difficult to compare the studies and extrapolate clinically relevant findings (115). In one sense, our findings were similar to those of Shamley et al. and Crosbie et al. who reported increased scapula upward rotation following mastectomy (111,117). However, an

important difference was that these differences were found specifically following mastectomy, not lumpectomy + RT, which limits comparison accuracy. In fact, Shamley et al found that scapula upward rotation was greater following mastectomy vs. lumpectomy + RT. Although Shamley et al. included healthy survivors in their study, kinematics were not compared between lumpectomy + RT and healthy survivors, or between the affected vs unaffected side as we performed in our study. Spinelli et al, in contrast, did not find a difference in scapulothoracic rotations between those treated with lumpectomy + RT as compared to those who had mastectomy + reconstruction and healthy women without a history of breast cancer but they did not perform within-subject comparisons. Together, these contrasting findings highlight the need for comparisons among similar treatment regimens as different oncologic and plastic surgeries, radiation treatments, and other cancer treatments may lead to diverse physiological and functional outcomes. Additionally, we did not control for PT or MD intervention for upper quadrant dysfunction. It is possible that that this could have skewed the results if such interventions changed shoulder kinematics in any way.

Although research is more prevalent demonstrating alterations in shoulder kinematics following mastectomy + ALND, we chose our population to include only lumpectomy + SLNB + RT as current research supports this treatment paradigm over more surgically invasive treatments for early-stage breast cancers (21). It is possible that our study was underpowered to detect kinematic differences in this population as many of them did not have shoulder pain. A stronger study design is warranted that includes breast cancer survivors with and without shoulder pain, or alternatively, the intentional inclusion of more aggressive treatment regimens such as breast RT + RLNR vs. breast RT alone as RLNR is a known risk factor for shoulder dysfunction (13). The lack of significant results in the kinematic portion of our study may also be to high variability, or simply that more significant differences do not exist.

Future studies could include electromyography to detect neuromuscular activation of the SA, PMaj, lower and middle trapezius, and upper trapezius to better understand muscle activation patterns that could affect ST kinematics. In fact, Shamley et al demonstrated that after a lumpectomy, the serratus anterior and pectoralis major had decreased muscle activity with upper extremity movement in the scapular plane (229). Brookham et al also demonstrated altered muscle activation patterns after breast cancer treatment (227). A future study could evaluate movement before surgery, after surgery, and then longitudinally after radiation to better understand shoulder kinematics, muscle activity, and function along the spectrum of breast cancer treatment.

Future research could also examine glenohumeral joint movement patterns to evaluate the stability and rotation of the humeral head in the glenoid. It is common for breast cancer patients (both following lumpectomy and radiation as well as following breast reconstruction that involves placement of the expander and/or implant under the pectoralis major) to have excess anterior mobility of the humeral head (RB, clinical practice). Pain with glenohumeral external rotation in these patients is often alleviated with the Jobe Relocation Test which involves a decrease in impingement symptoms with posterior glide of the humeral head in the glenoid (230). A tight or short pectoralis major can contribute to this due to the anterior pull of the tendon on the proximal humeral at its insertion, or by a tight or stiff pectoralis muscle due to the RT as well as subpectoral breast reconstruction technique. Anterior movement of the humeral head due to increase active and passive PMaj forces has been shown to decrease glenohumeral joint stability (231) but has not been validated in the breast cancer population.

A major limitation of the Flock of Birds and other 3D kinematic analyses using markers taped onto the skin is skin slip, or the inaccuracy of the sensor to specifically represent underlying joint motion due to movement of the skin and sensor on top of the joint (46,92). Efforts were made to minimize this including offset taping distally to minimize the cord pulling on the sensor. Bone pins or fluoroscopy are more reliable methods to capture joint movement, but we did not attempt these in an attempt to minimize invasiveness and radiation exposure in the oncology population. Speed of movement can also affect kinematics; we attempted to keep speed constant by verbally counting to 3 seconds for each repetition of arm elevation using a wall clock second hand each time, and by having each participant practice arm elevation 3 times in each plane prior to data collection. Other limitations included SC rotations. As the clavicle only has 2 linear points and 3 are needed to make a plane and coordinate system (214), a jig was used to obtain a third noncollinear landmark parallel and superior to the clavicle. Additional errors may have come from metal in the room as that can interfere with the electromagnetic Flock of Birds system (85) or from inconsistencies when digitizing the anatomical landmarks. Both AWS and lymphedema have the potential to affect shoulder kinematics (71,205), but neither were controlled for in this study.

Throughout this study, we chose to use linear regression instead of ANOVA due to the more robust nature of linear regression. Linear regression can account for additional sources of variation in the data such as the multiple potential covariates that we tested (up to 12 in 1 model) to see if they

affected the outcome. However, if all ANOVA assumptions are met, then linear regression should give the same results as ANOVA. Due to the multiplicity of potential covariates used, we decided to be consistent and use just linear regression throughout our statistical analyses. We did run 3-way repeated measures ANOVA to see if the results were consistent with our linear regression models. The ANOVA was run as affected/unaffected * rotation angle * plane of elevation. Resulting significant values were the same as those obtained by linear regression (with a significant main effect of angle but no significant interactions with treatment side or plane) for ST upward rotation, ST internal rotation, SC retraction, and SC elevation, and no significant main or interaction effects for ST posterior tilt or ST posterior rotation.

Specific Aim 3 : PSS and RT dose, PSS and EI

Penn Shoulder Score

The PSS total scores of breast cancer survivors were high, suggesting very good overall shoulder self-reporting with an average total score of 91.8 / 100. A high PSS total score signifies high function, low pain, and high satisfaction subscores. Harrington et al reported lower PSS scores (77.1 +/- 18) than in the current study (191). However, their study included breast cancer patients who had completed treatment less than 6 months prior to the study. At the stage of recovery that Harrington reported, we would expect the PSS to be lower as the participants are likely to still be healing from surgery, radiation and / or chemotherapy and may not have regained their pre-treatment activity and strength levels. In contrast, all participants in our study were at least 1-year post-RT, and our results reflect more chronic changes in function as compared to Harrington et al. Harrington's study population included 16 patients who had a mastectomy and 8 who had a lumpectomy, with only 2/8 having RT after lumpectomy. In contrast, all of our participants had lumpectomy + RT, which makes it difficult to compare outcomes among our distinct surgical and RT populations.

Our results were similar to previous research by Spinelli et al. who reported the PSS for breast cancer survivors at least 3 months after radiation and/or surgery (103). They reported average total score (+/- SD) of 85.3 (19.5), function 51.2 (11.7), satisfaction 7.9 (2.9), and pain 26.1 (5.8). Their population of breast cancer survivors was more diverse than in the present study as it included participants who had lumpectomy + RT as well as mastectomy + reconstruction and a few

participants with ALND. This suggests that overall, based on these 2 studies, that self-perceived shoulder function and satisfaction are high, and pain is low in breast cancer survivors.

However, when the PSS was further examined for its component questions, many breast cancer survivors do have difficulty with certain tasks. Almost 50% of participants said they had some difficulty placing a 1-gallon container on a shelf overhead without bending their elbows, and over 33% of participants said they could not place that container on a shelf at shoulder level without bending their elbow. One in 5 had some difficulty returning to their usual sport/hobby and 1 could not return to that activity at all. More than 25% reported some difficulty carrying groceries with the affected arm, sleeping on the affected side, and/or washing the middle of their back or hooking a bra with the affected arm. Therefore, the total PSS score may not accurately reflect specific functional problems that some breast cancer survivors experience. Additionally, future studies should include kinematic analysis with a weighted object as well as strength assessment.

This perspective of the PSS can guide healthcare providers to ask pertinent, targeted questions that can highlight impairments or functional limitations that can be addressed in rehabilitation. If we only look at the overall score of the PSS, which is common in clinical practice, it appears that breast cancer survivors have very high scores and look to be fully functional. However, examination of the individual questions reveals more subtle shoulder impairments that are necessary for ADLs. If we only look at the PSS total score, we may not recognize and therefore not address these impairments, and thus miss the opportunity to help breast cancer survivors regain full function of their upper extremity.

Future research should be aimed at identifying specific shoulder impairments and functional activities instead of concentrating on the total PSS score. Clinical use of specific functional questions rather than broad-based questionnaires may have better clinical application as they can more specifically identify functional impairments in breast cancer survivors, leading to referral to a physical therapist or other qualified professional to help the survivor regain full pain-free function after treatment. However, at this time, the PSS is one of a few validated tools available to evaluate shoulder function in breast cancer survivors, and the use of single or a few questions would also need to undergo rigorous validation to ensure reliability and accuracy.

Hypothesis 3.1 (PSS relationship to RT)

One of our main findings was that PMin and SA radiation doses were moderately and significantly correlated with PSS pain and satisfaction subscores, but none were significantly correlated with PSS total or PSS function subscore. This agrees with the findings of the PSS total and subscores just discussed. This is clinically important as clinicians often use the PSS total score alone, and do not look at component parts. To better understand the potential relationship between adjuvant RT and shoulder function, researchers need to better understand how and why pain was most consistently correlated with PMin V10-V40 values as well as SA mean dose, SA mean dose, V15 and V40 values. Our research suggests that those who had a higher percentage of the PMin and SA muscles exposed to at least 10Gy of radiation had an increased risk of shoulder pain. It is important however to remember that correlation is not causation, and that further research needs to be done to better understand these relationships.

For this reason, we also performed multiple linear regression. Although there were trends for the PSS total score to be lower in those with higher total RT dose, PMin mean dose, and PMin V30 values, these were not significant after correction for multiple testing and should be explored in future studies. PSS function score tended to be lower in those with higher total RT dose, PMin mean dose and PMin V30 doses but again, these were not significant after correction for multiple testing and should be explored in future studies. Additionally, PSS pain score tended to be higher in those with higher total RT dose and PMin V30 values. PSS total, function, and satisfaction scores were lower in those with higher PMin mean dose but higher in those with PMin V30 values, again not significant after correction for multiple testing. It is not clear why the PMin mean dose and V30 values would have opposite effects on the PSS values. It is possible that as these were trends, they are not significant. However, it warrants more research in the future so we understand any potential relationship that could improve shoulder function in breast cancer survivors.

A study by Johansen et al. found a statistically significant correlation between shoulder joint V15 dose and the Kwan's Arm Problem Scale (KAPS) score (r = .21, p = 0.003) in a cohort of 183 breast cancer survivors an average of 42 months after treatment(9). The KAPS includes patient-reported shoulder ratings of pain, stiffness, swelling, use of the arm, and numbness, and individual correlations of the V15 dose with these parameters ranged from .15 - .22 (significant at $p < 0.05$). In contrast to our study in which only 3 participants received RLNR, all participants had either a lumpectomy or mastectomy followed by adjuvant radiation to the breast or chest wall, supra- and infra-clavicular fossa, internal mammary lymph nodes, and axillary lymph nodes. Therefore, their

study participants were exposed to much more radiation than in our study. In contrast to Johansen's study, our research did not find any statistically significant correlations with PSS function or total score, only PSS pain and satisfaction scores with values ranging from $r = 0.34 – 0.47$ which were significantly higher correlations than what they found.

Also different than our study, Johansen et al measured forward flexion and abduction ROM with a goniometer but no kinematic analyses were performed, and tissue fibrosis was subjectively rated by an oncologist and a PT by palpating tissue stiffness and rating it from 0-4. They also found a statistically significant relationship between abduction ROM and V15 dose ($r = .18$, $p = .017$). This was done by treating abduction ROM as a binary variable, with those having $\geq 25°$ difference side to side having larger V15 doses. It is possible that this analysis type could have contributed to the significance of their findings. Johansen's radiation dose contouring also different from ours. They contoured the shoulder joint as a whole from the outer edges of the humerus, the coracoid process, and the acromion, but did not individually assess radiation dose to specific muscles. They concluded that their associations between RT and shoulder function were not strong, and that patient outcomes are likely related to surgery, chemotherapy, and other treatment components.

Marazzi et al studied 111 breast cancer survivors an average of 34 months after RT(195). They found a statistically significant negative correlation between a shoulder joint mean dose of 7Gy and DASH score ($p<0.001$). They also found a significant correlation of mean shoulder dose to DASH ($p=0.006$), age ($p <0.05$), and abduction ROM measured using a goniometer ($p=0.005$). They defined and contoured the shoulder for radiation dose analysis including the entire humeral head, glenoid, AC joint, and included ligaments. Every participant in their study had adjuvant RT to the breast and regional lymph nodes, which is known to have increased radiation exposure to the tissues (Lipps2017) and could explain why they found significant correlations of RT dose to DASH scores while our study did not. Additionally, it is possible that the DASH (as compared to PSS) was a more sensitive indicator of the relationship between RT dose and shoulder function.

Bazan compared radiation exposure to the shoulder and back in breast cancer survivors between 2 different radiation therapy techniques (3D conformal radiation therapy and intensity modulated radiation therapy) (232). They contoured the shoulder and back OARs inclusive of muscles, soft tissues, bones, and vasculature in the posterior neck, shoulder and upper arm region beginning from

2cm superior to the most cranial image to the most caudal image from the supraclavicular planning target volume. All participants received 50Gy in 25 fractions plus RLNR and 54% had Stage III disease, 72% had a mastectomy and 83% had an ALND as compared to our population which was all early stage (I or II), lumpectomy, and SLNB. They separated their radiation dose analysis into shoulder OAR and back OAR tissues as well as a combined shoulder + back OAR. (Bazan). The shoulder OAR included the PMaj and the back OAR included the SA but both back and shoulder volumes included many other muscles as well, in contrast to our study that contoured each muscle individually in an effort to isolate specific effects of those muscles with function. They performed V5-V50 analyses for each group of muscles using DVH analysis. Patient-reported function using the quick-DASH was only reported in those who presented for PT because of underlying impairments such as lymphedema or shoulder pain, which occurred in only 20% of their study population. It is therefore likely that their scores would reflect higher levels of shoulder dysfunction, and not appropriate for a correlation with radiation dose analysis.

Both Lipps and Wolfram used the same methodology to contour the PMaj muscle, and their volume of muscle will be different than ours as radiation of regional lymph nodes in their study exposes more of the pMaj and PMin to radiation(42,52). Our findings agreed with this. However, most participants in our study did not have RLNR, so our participants, on average, had less PMaj and PMin muscle exposed to RT than Lipps and Wolfram. The SA would also likely have more radiation exposure when a lower quadrant boost is used during RT, but Lipps and Wolfram did not analyze their findings using this treatment type (boost) as a predictor, or SA radiation dose as an outcome. Further sub analysis of our data could determine the additional dose of radiation to the PMaj, PMin, and SA using RLNR, boost, and breast RT dose analysis to better understand skeletal muscle RT exposure that may offer insight into muscle and shoulder function in breast cancer survivors.

As skeletal muscle radiation dose analysis is a relatively new area of research, no gold standard yet exists for measuring radiation dose to the soft tissues on the chest wall that lie within the radiation field. DVH analysis is an integral part of standard RT and is designed to maximize radiation dose to the area at risk and minimize dose to essential organs such as heart, lung, and contralateral breast. However, only a few previous studies have used DVH and general RT dose analysis to determine the effects of RT on shoulder joint and / or skeletal muscle. We decided to define the area of analysis using the inferior aspect of the cricoid cartilage as the most superior border and the bottom of the

radiation field as the most inferior border to maximize reliability between scans and among body morphologies, which is different than others previously and limits direct comparison. However, we believed that the use of anatomical landmarks adds strength to our study. This anatomical landmark is inclusive of RLNR as well as standard breast RT. We also decided to contour the PMaj, PMin, and SA individually as they each have the potential to have a unique impact on shoulder dysfunction based on their anatomical attachments to the clavicle, affecting SC movement, or scapula, affect ST movement, both of which can alter shoulder kinematics via the coupling mechanism as previously discussed.

Potential limitations of this technology include inconsistent contouring of the muscles. RAB consulted with radiologists at the University of Minnesota and utilized an online anatomy and imaging source recommended by those radiologists (Imaios). This software labels each muscle in CT scans, MRI scans, and anatomical drawings similar to those utilized in the radiation CT simulation scan. Another potential source of error was fusion of multiple scans for those participants who had multiple treatment plans. For example, scans can be performed using 'free breathing' or 'deep inspirational breath hold' for the breast, boost, and/or RLNR treatment plans. Each scan was overlayed on top of the others by a SE, a certified Radiation Dosimetrist, but it is possible that the muscles outlined in each scan were not 100% overlapped which could results in some, and likely minimal, error.

Another significant limitation of the radiation dose analysis is the lack of adjustment required to account for different fractionation regimens. This can be corrected by expressing the radiation dose as EQD2, or equivalent total dose expressed using a 2 Gy fraction dose. All radiation dose calculations can be corrected in this fashion using an equation that includes daily fraction dose, total dose, and alpha/beta ratio. Future analyses of this data and publications will include these adjustments (152). Consultation with Dr. Yuan, the treating radiation oncologist, suggests that these corrections will likely not change the overall findings or statistical analyses of this study.

Hypothesis 3.2 (PSS relationship to EI)

We were unable to prove our hypothesis that PMaj, PMin, and/or SA EI would be significantly related to with PSS as per Hypothesis 3.2. This may simply be because we did not find any significant differences in EI for any of the three muscles when comparing the affected and unaffected sides. As previously mentioned, B-mode US EI measures may not have been the most

accurate or sensitive measure to capture changes after surgery and RT. Recent research has demonstrated increased stiffness of the PMaj after breast cancer treatment using US elastography(42), and future research should further examine the relationship between US elastography, shoulder kinematics, and shoulder function.

Exploratory Analyses

Exploratory analyses were performed to determine the relationship between RT treatment plan and estimated absorbed radiation dose to the 3 muscles of interest. We found that those who had RLNR had significantly higher mean dose to the PMaj and PMin and a higher percentage of the PMaj and PMin were exposed to at least 30Gy (V30). These findings are similar to those of Lipps et al who were the only other researchers to specifically contour the PMaj and PMin, but who did not examine the SA(52).

We also found that those who had larger radiation boost doses (1250Gy vs 1000Gy) had larger PMaj V30 doses, meaning that they had a larger percent of the PMaj muscle receiving at least 30Gy. To the best of our knowledge, this is the first research to demonstrate the effect of RT boost on muscle. A total of 18/24 participants had a boost to the tumor bed, with 12 participants receiving a 1000Gy boost and 6 participants receiving a 1250Gy boost. Boost dose did not impact PSS score, but future research should determine if it affects SC or ST kinematics or even patient-reported shoulder scores, with study design specific to address this question.

Further exploration of boost location was performed as during analysis it was visually apparent that tumors that are located in the superior portion of the breast received boost RT to that area which overlays the pectoral muscles, as compared to tumors that are located in the inferior part of the breast that overlays more of the SA muscle. Medical treatment frequently classifies breast carcinoma in terms of quadrants: upper inner, upper outer, lower inner, and lower outer. For the purpose of our exploratory analysis we consolidated the data into upper vs lower quadrants. We found that those who had tumors in the lower half of the breast had increased SA mean RT dose and SA V30 values. Further research needs to be done to determine the effects of this on patient pain and/or function, especially as SA function is implicit in normal shoulder movement.

A strong correlation was found between the PMaj and PMin mean RT doses, which makes sense due to the anatomic location of the PMin under the PMaj. To the best of our knowledge, this is the

first time this has been shown in the literature. The PMaj has primarily been studied in the literature possibly due to its superficial location in the radiation field, but the PMin can affect shoulder posture and movement and should be included in future studies.

Breast size also has the potential to affect tissue volume exposed to radiation due to anatomical location of the breast on the chest wall, but to the best of our knowledge this has not yet been explored and breast size was not captured during our data collection, both subjective and in EPIC. This should be explored in future research.

Custom Questionnaire

The custom questionnaire utilized in this study was developed by RAB who has 20 years of experience working with cancer survivors, many of whom had adjuvant RT following lumpectomy. It was designed to capture the participants' perspectives and subjective symptoms that were not captured by the PSS or by medical records. As expected, the majority of participants (76.9%) experienced skin irritation in the radiation field during or after radiation (173). However, only 23.1% of the participants responded 'yes' to having discomfort or pain, but 42.3% reported having shoulder, arm, or chest wall symptoms within 1 week of this study, 38.5% complained of stiffness, and 30.8% complained of tightness and/or achiness. It is common practice for medical practitioners to ask if their patients have any shoulder pain, but 'stiffness, tightness, or achiness' are not commonly asked questions by many physicians (RAB, clinical experience). For example, one participant in the study was asked by the treating oncologist if she had any shoulder pain and responded no. When RAB interviewed this participant for the study, she admitted that she had 'stiffness in my chest wall', but not specifically 'pain' as asked. Despite the prevalence of subjective complaints such as stiffness or tightness, only 1-2 participants were each diagnosed with rotator cuff tendonitis or tear, shoulder strain, or shoulder impingement, again suggesting that these subtle functional impairments may be missed.

Additionally, symptom location was not isolated to the shoulder itself. Although 38.5% of participants complained of shoulder symptoms, 26.9% complained of symptoms in their arm and 19.2% complained of symptoms in their chest wall and/or elbow. Less frequently (3% - 7%), the breast, neck, axilla, and/or back were other symptom locations. Medical providers should ask their patients about these symptom locations, not just the shoulder, following adjuvant RT.

In terms of patient-reported symptom onset, more participants felt that RT and not surgery caused their symptoms. In those who felt that their symptoms started after RT, the majority of participants said it started acutely after RT or >12 months after, coinciding with the acute and late radiation effects known in the literature(150).

Lastly, only 2 participants stated they were diagnosed with AWS at some point since surgery (7.7%) but 5 participants were found to have AWS on the day of the study. None of those participants were aware that they had AWS. Due to human error, RAB only examined 16/26 (62%) of the participants for AWS in the study. The known prevalence of AWS following sentinel lymph node biopsy ranges from 11% - 58% (212), which fits with the partial data collection that we have. As AWS can affect shoulder kinematics, the examination for AWS should be included in every rehabilitation consult (71).

A limitation of our study included the risk of Type I error. As more factors were tested than were used in the final regression models for Aims 1-3 and the exploratory analyses, our statistics were subject to a Type I error that was partially controlled for using the q-value. These results should be tested in future studies specifically designed to test these covariates such as comparing similar groups on the basis of RT treatment type with all other treatments (e.g. endocrine therapy) held constant. Additionally, increasing sample size and decreasing variability could increase power in future studies.

Lastly, participants were asked if they received any medical treatment (MD or PT) following surgery and radiation. As 5 participants had PT and 1 had lymphedema therapy, it is possible that PSS values were higher / skewed positively due to the likely beneficial effect of PT on patient-reported shoulder function. This could make it more difficult to find significant effects of radiation on PSS as the range of PSS levels was small and skewed positively.

CHAPTER 7: CONCLUSION AND FUTURE DIRECTIONS

Breast cancer survivors have a high prevalence of shoulder dysfunction, but the etiology is not well understood. Lack of understanding contributes to inadequate diagnosis and treatment, which translates into decreased independence and/or increased pain/difficulty with ADLs and physical activities. Our goal for this dissertation was to better understand the high prevalence of shoulder dysfunction after breast cancer treatment by exploring the relationships among 3D shoulder

kinematics, tissue level morphology, radiation treatment dose analysis, and patient-reported shoulder outcomes.

We validated the use of B-mode US of the PMaj and PMin (intrarater reliability only) to examine skeletal muscle morphology in our healthy cohort. However, no significant differences were found in EI or CSA between the affected and unaffected side in our breast cancer survivor cohort. Potential reasons for this are discussed above, and future research should focus on ultrasound elastography that reflects stiffness, or other advanced imaging such as MRI that has better sensitivity to detect small changes in muscle morphology that may occur secondary to surgery and radiation.

Three-dimensional kinematic analysis revealed increased clavicle elevation during shoulder abduction and at the highest range during forward flexion. This is a common compensatory mechanism seen in other shoulder dysfunction populations adopted to facilitate normal shoulder motion. However, clinical relevance other than compensation strategy is not well understood. Future research aimed at examining shoulder strength (scapulothoracic muscles and rotator cuff) and glenohumeral accessory mobility could be performed, although it is possible that there is a strong subconscious protective component as well that could contribute to this shoulder shrug pattern.

One of the most clinical applicable findings of our study come from Aim 3, looking at the relationship between PSS and radiation treatment. Patient reported outcomes are commonly used in medical practice, often given to patients on an iPad with automated scoring performed by the program and a total score appearing on the medical provider's screen (PSS total score). Our research demonstrated that although average PSS total score was high, certain functional tasks are still difficult for a larger number of patients to perform. Almost 50% of participants had difficulty lifting a 1-gallon (such as milk) object on a shelf overhead without bending their elbows, and 38% were unable to lift a 1-gallon object to shoulder height. Additionally, >1/4 participants had difficulty sleeping on their affected side and carrying a bag of groceries, confirming functional limitations after lumpectomy and RT.

If medical providers only look at the total PSS score, they may not recognize or address these subtle yet important functional difficulties that exist for some patients. Patients complained of

stiffness and tightness, not just pain. They also complained of problems not just with their shoulders, but also their chest, arm, and elbow. As a result of these findings, we recommend that medical providers intentionally ask the specific questions pointed out in this report as being problematic in addition to the patient reported outcome (which is often necessary for medical insurance purposes), as well as asking about arm, elbow, and chest wall issues and not focus soley on the shoulder. Further research should be conducted to validate the use of these few pointed questions in order to best help our breast cancer patients return to pain-free ADLs.

We found a trend for the PMin mean dose to negatively affect total PSS score as well as function and pain scores, but adjustment for multiple testing with the linear regression made this finding not statistically significant. As a similar trend existed for PMin V30 dose to increase PSS scores, which contradicts the other finding, the validity of these results is questionable.

Significant correlations between the volume of the PMin affected by radiation (V10, V15, V20, V30) and the PSS pain subscale as well as between the SA V15 , V40, and mean doses and pain warrant further exploration. Future studies should be designed and powered to determine if there is a minimum radiation dose that is associated with shoulder dysfunction.

Exploratory analyses demonstrated significant relationships between RLNR and radiation dose to the PMaj and PMin as well as boost dose and PMaj radiation dose. Additionally, we discovered a relationship between tumor location and dose to the SA. Future research needs to expand on the significant relationships between RLNR and radiation dose to the PMaj and PMin as well as the effect of boost dose and boost locations on muscle dose as well as muscle and shoulder function.

In summary, if we can show that radiation dose affects shoulder / chest wall / arm function and/or pain, then skeletal muscle radiation dose analysis can be considered a new biomarker and added to the traditional organs at risk analysis during radiation planning. This information can then be used to improve best practice by facilitating the referral of those at risk of shoulder problems to physical therapy, or at a minimum, to educate the patient of their risk and validate future concerns that are often overlooked. This type of research can be applied to other oncology populations such as head and neck cancers, where treatment often includes surgery and radiation, and patients often report neck and/or shoulder dysfunction. It can also be expanded to explore and better understand the role of radiation in lymphedema risk.

As breast conservation therapy (lumpectomy) + RT is likely to continue to be the treatment of choice for early-stage breast cancers, and chronic shoulder dysfunction is prevalent in this population and significantly affects QOL, it is important that we continue to learn more about the impact of adjuvant RT on shoulder function. As medical providers we are uniquely positioned to help breast cancer survivors thrive during and after treatment. We need to continue to investigate changes that can occur secondary to cancer treatment. Survival is foremost, but *quality* of survival is important as well.

Future research should include measurement timepoints that can better isolate pre-existing shoulder comorbidities as well as surgical and radiation effects. Measurements should be made both pre- and post- surgically as well as before and at least 2 years after RT. Clinically relevant outcomes such as shoulder ROM and patient-reported shoulder function should be included. Research methodologies designed to capture tissue-level changes such as a more sensitive measurement of tissue fibrosis or stiffness should be used. A standardized method of radiation dose analysis should be developed and/or agreed upon, so that we can compare information between studies. Risk analysis should be performed to determine who is at high risk of shoulder dysfunction. By better understanding the long- and short-term sequelae of cancer treatment, we can intervene proactively, treat effectively, and help provide comprehensive multidisciplinary medical care focused not just on breast cancer survivorship, but on quality of life.

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APPENDIX A

The Penn Shoulder Score, Part 1: Pain and Satisfaction Subscales

Please circle the number closest to your level of pain or satisfaction	Office Use Only
Pain at rest with your arm by your side: 0 1 2 3 4 5 6 7 8 9 10 No pain — Worst pain possible	_____ (10 – # circled)
Pain with normal activities (eating, dressing, bathing): 0 1 2 3 4 5 6 7 8 9 10 No pain — Worst pain possible	_____ (10 – # circled) (Score 0 if not applicable)
Pain with strenuous activities (reaching, lifting, pushing, pulling, throwing): 0 1 2 3 4 5 6 7 8 9 10 No pain — Worst pain possible	_____ (10 – # circled) (Score 0 if not applicable)
Pain score:	=____/30
How satisfied are you with the current level of function of your shoulder? 0 1 2 3 4 5 6 7 8 9 10 Not satisfied — Very satisfied	____/10 (# circled)

The Penn Shoulder Score: Function Subscale

Please circle the number that best describes the level of difficulty you might have performing each activity	No difficulty	Some difficulty	Much difficulty	Can't do at all	Did not do before injury
1. Reach the small of your back to tuck in your shirt with your hand	3	2	1	0	X
2. Wash the middle of your back/hook bra	3	2	1	0	X
3. Perform necessary toileting activities	3	2	1	0	X
4. Wash the back of opposite shoulder	3	2	1	0	X
5. Comb hair	3	2	1	0	X
6. Place hand behind head with elbow held straight out to the side	3	2	1	0	X
7. Dress self (including put on coat and pull shirt off overhead	3	2	1	0	X
8. Sleep on affected side	3	2	1	0	X
9. Open a door with affected arm	3	2	1	0	X
10. Carry a bag of groceries with affected arm	3	2	1	0	X
11. Carry a briefcase/small suitcase with affected arm	3	2	1	0	X
12. Place a soup can (1-2 lb) on a shelf at shoulder level without bending elbow	3	2	1	0	X
13. Place a one gallon container (8-10 lb) on a shelf at shoulder level without bending elbow	3	2	1	0	X
14. Reach a shelf above your head without bending your elbow	3	2	1	0	X
15. Place a soup can (1-2 lb) on a shelf overhead without bending your elbow	3	2	1	0	X
16. Place a one gallon container (8-10 lb) on a shelf overhead without bending your elbow	3	2	1	0	X
17. Perform usual sport/hobby	3	2	1	0	X
18. Perform household chores (cleaning, laundry, cooking)	3	2	1	0	X
19. Throw overhand/swim/overhead racquet sports (circle all that apply to you)	3	2	1	0	X
20. Work full-time at your regular job	3	2	1	0	X

SCORING
Total of columns = ____ (a)
Number of Xs × 3 =____(b), 60 – ____(b) = ____ (c) (if no Xs are circled, function score = total of columns)
Function Score = ____(a) ÷ ____(c) = ____ × 60 ____/60

APPENDIX B

Healthy Demographics

Please complete the survey below.

Thank you!

This survey will let us know a little bit more about you. Thank you for taking the time to fill it out. Do not hesitate to reach out with any questions: rbraudy@umn.edu.

What is today's date?

_____
(mm/dd/yyyy)

What is your first and last name?

_____

What is your date of birth?

_____
(mm/dd/yyyy)

What is your gender?

◯ Female
◯ Male
◯ other
◯ choose not to disclose

If other, please explain. This is optional.

_____

What is your race / ethnicity? (Choose all that apply)

☐ American Indian or Alaskan Native
☐ Asian
☐ Native Hawaiian or other Pacific Islander
☐ Black or African American
☐ White
☐ Hispanic / Latino of any race
☐ Two or more races
☐ I do not want to answer
☐ Unknown
☐ Other

If other, please explain. This is optional.

_____

What is your height?

_____

What is your weight?

_____

Do you have a history of current or past shoulder or neck pain?

◯ No
◯ Yes

Please explain.

_____

What is your email? _____

What is your phone number? _____

What is your address? _____

Thank you for filling out this survey! We appreciate your time and effort.

APPENDIX C

Participant Characteristics

Please complete the survey below to help us better understand more about you, your medical history, and how your shoulder is feeling.

Thank you!

Name	_____
The purpose of this questionnaire is to learn more about how your arm/shoulder are feeling as well as about your breast cancer radiation treatment. This information may help us better understand any difficulties with your shoulder or arm that you may be having. Please fill out this form to the best of your ability and let us know if you have any questions. Thank you!	
Today's Date	_____
Zip Code	_____
Date of birth:	_____
Gender	◯ Female ◯ Male ◯ Other ◯ Choose not to disclose
Race (check all that apply)	☐ American Indian or Alaskan Native ☐ Asian ☐ Native Hawaiian or Other Pacific Islander ☐ Black or African American ☐ White ☐ Hispanic/Latino of any race ☐ Two or more races ☐ I do not want to answer ☐ Unknown ☐ Other (please specify)
If other: please specify	_____
Handedness:	◯ Right ◯ Left
Did you have any skin irritation during or after radiation (in the radiation area on your chest wall , armpit, and / or breast)?	◯ Yes ◯ No
How would you describe those symptoms/skin irritation? (Choose all that apply.)	☐ Red ☐ Itchy ☐ Rash / bumpy ☐ Blister ☐ My skin peeled ☐ Sunburn ☐ Other

Please describe further:	_____
How would you describe the severity of those symptoms?	◯ Mild ◯ Moderate ◯ Severe
Have you had any current or past problems with your affected arm, shoulder, or chest wall following surgery and radiation (such as pain, stiffness, weakness, difficulty doing normal activities with that arm, etc)?	◯ Yes ◯ No
Please indicate any of the following symptoms that you may feel (or used to feel) in your chest wall, shoulder, or arm on the side that had surgery and radiation. (Choose all that apply):	☐ Discomfort/Pain ☐ Stiffness ☐ Tightness ☐ Weakness ☐ Achiness ☐ Heaviness ☐ Fullness ☐ Swelling ☐ Numbness/Tingling ☐ Pulling ☐ Stinging ☐ Stabbing ☐ Tenderness ☐ Other (Please Specify) ☐ I have never had any shoulder symptoms or problems since surgery and radiation. ☐ I did have some issues but do not anymore.
Please tell us more.	_____
On a scale of 1-10, how would you rate your arm/shoulder/chest wall symptoms over the last week? (0 = no symptoms, 10 = significant symptoms)	◯ 0 ◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10
Where do you feel the symptom? (Choose all that apply)	☐ Shoulder ☐ Chest wall ☐ Breast ☐ Arm ☐ Elbow ☐ Wrist ☐ Hand ☐ Fingers ☐ Neck ☐ Other (Please specify)
If other, please specify:	_____

When do you feel your symptoms?	◯ At rest / when I am not moving ◯ All the time ◯ Only with certain movements
Are there certain movements that make your symptoms worse?	◯ yes ◯ no
What movements or activities make your symptoms worse?	_____
If reporting symptoms: what started your symptoms? Please check all the apply.	☐ Cancer surgery ☐ Radiation ☐ Trauma ☐ Fall on shoulder or arm ☐ "Wear and tear" ☐ I don't know why it started bothering me ☐ Other (please briefly describe)
If you replied 'Other', please explain.	_____
How long after radiation therapy ended did your symptoms begin?	☐ Immediately ☐ < 3 months after ☐ 3-6 months after ☐ 6-9 months after ☐ 9-12 months after ☐ >12 months after ☐ I am not sure how long after
Please add any details to better explain how / when your symptoms started after radiation therapy.	_____
How long ago did your symptoms begin?	◯ < 1 month ◯ 1-2 months ◯ 2-3 months ◯ > 3 months
Please provide number of months:	_____
Do your symptoms stop you from doing anything? Either daily activities or recreation/sports?	◯ Yes ◯ No
What do your symptoms stop you from doing, or what is more difficult to do because of your symptoms?	_____
What is your Occupation?	_____
Have you had medical treatment (seen a doctor or physical therapist) for your symptoms?	◯ yes ◯ no

What kind of medical treatment have you had? Please include information about any imaging (Xray, MRI), physical therapy, etc. that you had or are currently having.	_____
As part of your job or recreational activities, are you required to do an activity over and over (repetitive)?	◯ Yes ◯ No
As part of your job or recreational activities, do you use your arms overhead?	◯ Yes ◯ No
Have you ever smoked?	◯ Yes ◯ No
Are you a current smoker?	◯ Yes ◯ No
If yes, how many cigarettes per day?	_____
Have you ever been told by a medical provider that you have any of the following? (Choose all that apply)	☐ Lymphedema of the arm or chest wall ☐ Rotator cuff tendonitis/tendinopathy ☐ Rotator cuff tear ☐ Labral (shoulder cartilage) injury or tear ☐ Shoulder strain ☐ Shoulder impingement ☐ Shoulder bursitis ☐ Shoulder dislocation ☐ Fracture of the humerus (upper arm), shoulder blade or collar bone ☐ Shoulder surgery ☐ Neck Injury ☐ Scoliosis ☐ Axillary web syndrome / cording ☐ Other shoulder, neck, elbow, or hand/wrist injury or problem (please specify) ☐ I have never been told I have any of these problems.
Please tell us more. If you have had imaging (Xray, MRI), what did it show? If you had physical therapy, for how long?	_____
If other: please specify	_____
Menopausal Status:	◯ Premenopausal ◯ Postmenopausal ◯ Don't know
Are you taking (or have you taken) a hormone inhibitor/blocker as part of your cancer treatment?	◯ Yes ◯ No
Please provide as much information as you have on the name of the medication, dose, and how long you have taken it (or how long you took it if you have stopped).	_____

If you have comments you would like to make about anything on this questionnaire, or anything else regarding your history with breast cancer or shoulder problems, please let us know. Thank you for participating in this research study! Your time is much appreciated-

_____

APPENDIX D

Participant Log for Studies Recruiting M Health Fairview Patients

Record ID / REDCap ID

_____

Field in REDCap template that isn't visible to the end user and can't be changed, but will identify the use of the template in the data for identifying that this is a study associated with Fairview
DO NOT MAKE ANY CHANGES TO THIS FIELD

_____

OnCore Protocol Number 2022LS024

_____

Please note that this instrument includes identifiers, so it should be protected and managed per the Privacy Act and institutional policiesfor handling PHI.

In User Rights, you should restrict access to this instrument to those who should be allowed to see the identifiers and, for those to whom you give access to export data, exclude fields marked as identifiers except for those for whom doing that is acceptable. (People can export data from instruments that they do not have access to, so don't give access to exporting data to anyone who should not see any of the data on this instrument.) Instructions for using this instrument:

In order to get a full picture of M Health Fairview patients who are involved in research, you are requested to use this instrument in your REDCap project and fill it out for each participant in your study. Through the use of this form you understand that the data collected will be made available in the AHC-IE for reporting purposes.

Before entering any data into this project, do a one time edit of the "OnCore Protocol Number" field to set the default value to the correct OnCore Protocol Number for your study. This is done in the "Action Tags / Field Annotation" section of the Edit Field window.

In order to make your data entry job easier, you might be able to modify this form to pull some of the data in from other forms. To do this, you can edit a field and put @DEFAULT='[other_field_name]' in the "Action Tags / Field Annotation (optional)" section of the edit field window, replacing "other_field_name" with the name of the field on another form that contains this data.

If your project is longitudinal, then you will need to include the name of the event that the other instrument is in, but only if it is in a different event than this instrument is in. If you need to do this, it would be @DEFAULT='[event_arm_1][other_field_name]' replacing "event_arm_1" with the unique event name, which you can get from the page where you define your events. (There should be no space between the ending square bracket of the event name and the opening square bracket of the field name.)

Do NOT change any of the options in the multiple choice fields. This needs to be the same in all projects that are using this instrument in order to be able to combine the data from multiple projects.

Subject ID

_____

Is this participant a patient recruited from M Health Fairview?

- ◯ Yes
- ◯ No
- ◯ Unknown

M Health Fairview MRN (if known)

_____

First Name

_____

Middle Name or Initial	_____
Last Name	_____
Suffix	_____
Birthdate	_____
Phone Number	_____
Zip Code	_____
Gender	◯ Female ◯ Male ◯ Unspecified ◯ Unknown
Was this person ever enrolled in this study?	◯ Yes ◯ No
Initial Consent Date	_____

APPENDIX E

Breast Cancer Survivorship Research Summary

- 1 study visit at the University of Minnesota Cancer Survivorship and Lymphology Lab that will last ~2 hours.
- During the visit, we will test how your shoulder and arm move using sensors taped to your skin, and we will use ultrasound to learn more about chest wall muscle quality.
- You will be asked to fill out 2 email questionnaires
- You will receive $40 reimbursement for your time plus parking
- Your participation may help improve care for future breast cancer survivors with shoulder pain

Contact Us

Renata Braudy: rbraudy@umn.edu

University of Minnesota (East Bank) Cancer Survivorship and Lymphology Research Laboratory
Room 320
Children's Rehabilitation Center
426 Church Street SE
Minneapolis, MN 55455

UNIVERSITY OF MINNESOTA
Driven to Discover℠

Breast Cancer Survivorship Research

UNIVERSITY OF MINNESOTA

Dr. Linda Koehler, PhD, PT, CLT-LANA
Renata Braudy, PT, MS, MA, OCS, CLT, PhD Candidate

Shoulder Function after Breast Cancer

We are conducting a research study to better understand why some people have shoulder pain, stiffness, and / or decreased range of motion after surgery and radiation for breast cancer.

The information we learn from this research can help us identify and treat similar shoulder issues better in the future.

Up to 49% of breast cancer survivors have difficulty using their shoulder, and 64% have pain, swelling, or decreased shoulder motion ... 10 years after surgery.

The research study involves 1 visit to our Cancer Survivorship and Lymphology Research Laboratory on the East Bank of the University of Minnesota. You will also be emailed 2 questionnaires.

What will I need to do?

Shoulder Assessment

We will place 3-4 sensors (the size of a fingernail) on your arm, shoulder, and chest. You will be asked to move your arm overhead while a computer records how you are moving. This will be repeated using your other arm; it will take approximately 1 hour.

Ultrasound

We will perform an ultrasound of 3 chest wall muscles while you lay on your back. Both sides will be imaged. This will take less than 1 hour and will give us information about muscle size and scar tissue content.

Questionnaires

We will send you 2 questionnaires to fill out online at your convenience. One questionnaire asks about your medical history. The second one asks questions specific to your shoulder, such as if you have any pain or difficulty using it during daily activities since surgery and radiation therapy.

Appendix F

A collaboration among the University of Minnesota,
University of Minnesota Physicians and Fairview Health Services

To Whom it May Concern-

We invite you to participate in a research study about shoulder function after surgery and radiation for breast cancer. I am a co-investigator for this study, along with Renata Braudy, PT, a PhD candidate at the University of Minnesota, and Dr. Linda Koehler and Dr. Paula Ludewig, who are Faculty in the PhD Program in Rehabilitation Science at the University of Minnesota.

The purpose of this study is to learn more about changes that can occur after treatment that can affect how your shoulder feels or moves. You are eligible to participate in this study because you received these treatments, with radiation therapy specifically under my care.

Participation in this study will involve one visit to the University of Minnesota (East Bank, Cancer Survivorship and Lymphology Research Laboratory). This visit will take approximately 2-3 hours and will involve 2 main parts. First, we will use sensors taped to your skin to how your shoulders move while you raise your arm. Second, we will perform ultrasound to look at 3 muscles on your torso that are within the radiation field. There are also 2 questionnaires we will ask you to fill out. You will be compensated $40 plus parking for your time. A study flyer is included in this mailing with more information.

Your participation in this study is completely voluntary. If you choose to participate you may choose to discontinue participation at any time. The information collected in this study will not be part of your medical records and will not impact any other medical care you are currently receiving.

If you are interested in participating in this study, or would like more information or have questions, please contact Ms. Braudy at rbraudy@umn.edu or call 612-301-9539.

Thank you for your time and consideration.

Sincerely,

Dr. Yuan

Ms. Braudy

Dr. Ludewig

Dr. Koehler

mhealthfairview.org

Approved for use by UMN IRB
Effective on 11/9/2022
IRB Study Number: STUDY00016427

APPENDIX G

Consent Form (includes HIPAA Authorization)

Title of Research Study*: Postoperative Radiotherapy Breast Cancer Treatment: Musculoskeletal and Functional Implications*
Investigator Team Contact Information: *Linda Koehler, PhD, PT*
For questions about research appointments, the research study, research results, or other concerns, call the study team at:

Investigator Name: Linda Koehler, PhD, PT Investigator Departmental Affiliation: Rehabilitation Medicine Phone Number: 612-626-1502 Email Address: koeh0139@umn.edu	Study Staff: Renata Braudy, PT, MS, MA, OCS, CLT, PhD Candidate Phone Number: na Email Address: rbraudy@umn.edu

Supported By: This research is supported by ***American Physical Therapy Association Promotion of Physical Therapy (PODS) I Scholarship and the Council of Graduate Studies (COGS) at the University of Minnesota***.

Key Information About This Research Study

The following is a short summary to help you decide whether or not to be a part of this research study. More detailed information is listed later on in this form.

What is research?

Doctors and investigators are committed to your care and safety. There are important differences between research and treatment plans:

- The goal of research is to learn new things in order to help groups of people in the future. Investigators learn things by following the same plan with a number of participants, so they do not usually make changes to the plan for individual research participants. You, as an individual, may or may not be helped by volunteering for a research study.
- The goal of clinical care is to help you get better or to improve your quality of life. Doctors can make changes to your clinical care plan as needed.

Research and clinical care are often combined. One purpose of this informed consent document is to provide you clear information about the specific research activities of this study.

Why am I being asked to take part in this research study?

We are asking you to take part in this research study because you had surgery and radiation for breast cancer more than one year ago at the University of Minnesota / M Health Fairview.

What should I know about a research study?

- Someone will explain this research study to you.
- Whether or not you take part is up to you.
- You can choose not to take part.
- You can agree to take part and later change your mind.
- Your decision will not be held against you.
- You can ask all the questions you want before you decide.

Why is this research being done?

The purpose of this research is to better understand why some people have shoulder problems after surgery and radiation for breast cancer. Shoulder pain, stiffness, and/or weakness are common side effects of breast cancer treatment but are not well understood. This study will give us more information about why shoulder problems might occur, so that in the future we can identify and treat them better. The results of this study may help with the development of future rehabilitation programs designed to better identify and treat shoulder pain after breast cancer treatment.

How long will the research last?

We expect that you will be in this research study for 2-3 hours after the informed consent is completed. The date and time of the study will be set up at a convenient time for you. The ultrasound portion of the exam will take 30-60 minutes, and the 3-dimensional shoulder exam will take approximately 1 hour. Two questionnaires will be mailed to you that will take approximately 10-15 minutes to fill out.

What will I need to do to participate?

You will be asked to participate in 1 study visit that will take 2-3 hours, plus answer questionnaires that will be emailed to you. During the study visit, we will use sensors taped to your arm and chest to learn how your shoulder moves when you raise your arm overhead. We will also perform an ultrasound of 3 chest wall muscles on both sides of your body.

More detailed information about the study procedures can be found under ***"What happens if I say yes, I want to be in this research?"***

Is there any way that being in this study could be bad for me?

There are no significant risks associated with this study, and the measurements are non-invasive. If you have shoulder pain when you move your arm, you can stop at any time. It is possible that the questionnaires may remind you of your cancer treatment and make you feel emotional. There is a small risk of loss of confidentiality, but this will be minimized by using secure data storage and by assigning each participant a non-identifiable study number that has approved password protection required for access.

You will be offered a separate authorization form that permits use of University of Minnesota Email ("Unsecured Email Correspondence") in place of encrypted emails for communication, ie scheduling. You do not have to sign the Unsecured Email Correspondence form in which case the study staff will communicate via encrypted email only.

More detailed information about the risks of this study can be found under ***the "What happens to the information collected for the research?" section***.

Will being in this study help me in any way?

We cannot promise any benefits to you or others from your taking part in this research. However, possible benefits include:

If you have shoulder pain and have not received medical care for it, we will discuss whether a referral to a physician or to physical therapy would be helpful to you. Additionally, some of this information may help guide physical therapy treatment for any shoulder pain or discomfort you may have. The information learned from this study can also help guide future medical treatment of shoulder problems after breast cancer treatment.

What happens if I do not want to be in this research?

There are no known alternatives, other than deciding not to participate in this research study.

Detailed Information About This Research Study

The following is more detailed information about this study in addition to the information listed above.

How many people will be studied?

We expect about 30 people here will be in this research study out of 40 people in the entire study nationally.

What happens if I say "*Yes, I want to be in this research*"?

If you decide to participate in this research, after signing this informed consent and HIPPA form, you will be asked to come to the Cancer Survivorship and Lymphology Research Lab in the Children's Rehab Building on the East Bank of the University of Minnesota. Renata Braudy, PT (study staff) will meet you and get measurements described in this document at that time. Your total time commitment that day will be 2 hours and you will be compensated $40 plus parking for your participation.

During your study visit, we will perform 2 measurements: one to learn how your arm/shoulders move during daily activities, and the other to learn more about shoulder muscles in your shoulder and chest wall on both your operated and non-operated sides.

The measurement that helps us better understand how your shoulder moves is called 3-dimensional kinematic analysis, or 3D kinematics. We will place 4 small sensors on your chest, shoulder, and arm that help us measure how the individual bones move while you raise your arm in 2 different directions.

Ultrasound will be used to 'see' 3 muscles in your chest wall on both the affected and unaffected sides. This information will tell us the size of your muscles as well as if and how much scar tissue is present.

Your participation in this study also grants study staff access to your pre-radiation CT simulation scans. These scans will be used to learn how much radiation was delivered to the muscles we are examining under ultrasound.

Two questionnaires will be emailed to you. The first will tell us more about your cancer treatment including relevant background information (radiation experience, if you have shoulder pain, etc). The second questionnaire will ask about any shoulder pain or discomfort that you may be experiencing.

All of the items listed above are for research purposes only and do not constitute standard care.

What happens if I say "Yes", but I change my mind later?

If you take part in this research study, and want to leave, you should tell us. Your choice not to be in this study will not negatively affect your right to any present or future medical care, your academic standing as a student, or your present or future employment.

We will make sure that you stop the study safely. We will also talk to you about follow-up care, if needed.

If you stop being in the research, information about you that has already been collected may not be removed from the study database. In that case, the data that we were able to collect may be used in the study analysis, but only as de-identified data.

Can I be removed from the research?

It's possible that we will have to ask you to leave the study before you finish it. If this happens, we will tell you why. We will also help arrange other care for you, if needed.

Will it cost me anything to participate in this research study?

Taking part in this research study will not lead to any costs to you.

Will being in this study help me in any way? (Detailed Benefits)

We cannot promise any benefits to you or others from your taking part in this research. However, your participation may help others in the future. For example, improved understanding of why some people have shoulder problems after breast cancer surgery and radiation can lead to improved identification and treatment of their shoulder problems.

What happens to the information collected for the research, including my health information?

We try to limit the use and sharing of your information, including research study records, any medical records and any other information about you, to people who have a need for this information. But we cannot promise complete confidentiality.

Overview

Consent Form (includes HIPAA Authorization)

If you participate in this study, your information, including your health information, will be used and shared for purposes of conducting this research. As described later in this Consent Form, your information may also be used and shared for publishing and presenting the research results, future research, and any optional elements of the research you agree to in this Consent Form, which may include creating audio and video recordings of you. If you sign this Consent Form, you are giving us permission to use and share your health information for these purposes, and if we are using your medical records, you are giving permission to any health care providers who are treating you to share your medical records with us.

What health information will be made available?

Health information about you to be used and shared for the research includes those items checked by the research team below:

☒ Your medical records, which may include records from hospital and clinic visits, emergency room visits, immunizations, medical history and physical exams, medications, images and imaging reports, progress notes, psychological tests, EEG/EKG/ECHO reports, lab and pathology reports, dental records and/or financial records. These records may be used and shared for as long as this research continues.

☒ Information collected as part of this research study, including research procedures, research visits, and any optional elements of the research you agree to, all as described in this Consent Form. This information might not be part of your medical record, and may include things like responses to surveys and questionnaires, and information collected during research visits described in this Consent Form.

What about more sensitive health information?

Some health information is so sensitive that it requires your specific permission. If this research study requires any of this sensitive information, the boxes below will be marked and you will be asked to initial to permit this information to be made available to the research team to use and share as described in this Consent Form.

☐ My drug & alcohol abuse, diagnosis & treatment records _____ (initial)

☐ My HIV/AIDS testing records _____ (initial)

☐ My genetic testing records _____ (initial)

☐ My mental health diagnosis/treatment records ____ (initial)

☐ My sickle cell anemia records _____ (initial)

Who will access and use my health information?

If you agree to participate in this study, your information will be shared with:

- The University of Minnesota research team and any institutions or individuals collaborating on the research with us;

- Others at the University of Minnesota and M Health/Fairview who provide support for the research or who oversee research (such as the Institutional Review Board or IRB which is the committee that provides ethical and regulatory oversight of research at the University, systems administrators and other technical and/or administrative support personnel, compliance and audit professionals (Such as the Quality Assurance Program of the Human Research Protection Program (HRPP)) , individuals involved in processing any compensation you may receive for your participation, and others);

- The research sponsor(s), any affiliates, partners or agents of the sponsor(s) involved in the research, organizations funding the research, and any affiliates, partners or agents of the funding organization(s) involved in the research;

[Foundation for Physical Therapy Research]

- Organizations who provide accreditation and oversight for research and the research team, and others authorized by law to review the quality and safety of the research (such as U.S. government agencies like the Food and Drug Administration, the Office of Human Research Protections, the Office of Research Integrity, or government agencies in other countries); and

- Organizations that process any payments that may be made to you for participating in this study, and any other individuals or organizations specifically identified in this Consent Form.

Additional sharing of your information for mandatory reporting

If we learn about any of the following, we may be required or permitted by law or policy to report this information to authorities:

- Current or ongoing child or vulnerable adult abuse or neglect;
- Communicable, infectious or other diseases required to be reported under Minnesota's Reportable Disease Rule;
- Certain wounds or conditions required to be reported under other state or federal law; or
- Excessive use of alcohol or use of controlled substances for non-medical reasons during pregnancy.

How will my information be used in publications and presentations?

We may publish the results of this research in scientific, medical, academic or other journals or reports, or present the results at conferences. Information that makes it easy to identify you (such as your name and contact information, SSN and medical records number) will not be part of any publication or presentation. If you have an extremely unique or rare condition that is not shared by many others, it is possible that some people may be able to determine your identity even without these identifiers.

What will be done with my data when this study is over?

We will use and may share data and/or specimens for future research. They may be shared with researchers/institutions outside of University of Minnesota. This could include for profit companies. We will not ask for your consent before using or sharing them. We will remove identifiers from your data and/or specimens, which means that nobody who works with them for future research will know who you are. Therefore, you will not receive any results or financial benefit from future research done on your specimens or data.

If you leave the study, you can ask to have the data collected about you removed. You can also ask us to remove information that identifies you from the data. This may not be possible if data have already been shared.
Please indicate whether you will allow the identifiable data to be used for future research by putting your initials next to one of the following choices:

_____ (initials) NO, my identifiable data may not be used for future research. They may be used for this study only.

_____ (initials) YES, my identifiable data may be used for other future research studies

Do I have to sign this Consent Form and give my permission to make my information, including my health information, available for use and sharing?

No, you do not have to sign this Consent Form. But if you do not sign, you will not be able to participate in this research study. Treatment available outside of the study, payment for such treatment, enrollment in health insurance plans and eligibility for benefits will not be impacted by your decision about signing this Consent Form.

Does my permission for making my health information available for use and sharing ever expire?

No, there is no expiration date.

May I cancel my permission for making my health information available for use and sharing?

Yes. You may cancel your permission at any time by writing to the researcher at the address on the first page of this Consent Form. If you cancel your permission, you will no longer be in the research study. You may also want to ask someone on the research team in canceling will affect any research related medical treatment. If you cancel your permission, any health information about you that was already used and shared may continue to be used and shared for the research study and any optional elements of the study to which you agree in this Consent Form.

What happens to my health information after it is shared with others?

When we share your information with others as described in this Consent Form, privacy laws may no longer protect your information and there may be further sharing of your information.

Will I be able to look at my records?

It is possible that the research team may not allow you to see the information collected for this study. However, you may access any information placed in your medical records after the study is complete.

Will I receive research test results?

Most tests done on samples in research studies are only for research and have no clear meaning for health care. If the research with your identifiable information or samples gives results that do have meaning for your health, the investigators will contact you to let you know what they have found.

Will anyone besides the study team be at my consent meeting?

You may be asked by the study team for your permission for an auditor to observe your consent meeting. Observing the consent meeting is one way that the University of Minnesota makes sure that your rights as a research participant are protected. The auditor is there to observe the consent meeting, which will be carried out by the people on the study team. The auditor will not document any personal (e.g. name, date of birth) or confidential information about you. The auditor will not observe your consent meeting without your permission ahead of time.

Whom do I contact if I have questions, concerns or feedback about my experience?

This research has been reviewed and approved by an IRB within the Human Research Protections Program (HRPP). To share feedback privately with the HRPP about your research experience, call the Research Participants' Advocate Line at 612-625-1650 (Toll Free: 1-888-224-8636) or go to z.umn.edu/participants. You are encouraged to contact the HRPP if:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You want to talk to someone besides the research team.
- You have questions about your rights as a research participant.
- You want to get information or provide input about this research.

Will I have a chance to provide feedback after the study is over?

The HRPP may ask you to complete a survey that asks about your experience as a research participant. You do not have to complete the survey if you do not want to. If you do choose to complete the survey, your responses will be anonymous.

If you are not asked to complete a survey, but you would like to share feedback, please contact the study team or the HRPP. See the "Investigator Contact Information" of this form for study team contact information and "Whom do I contact if I have questions, concerns or feedback about my experience?" of this form for HRPP contact information.

What happens if I am injured while participating in this research?

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to you or your insurance company. Under some circumstances the sponsor of the study will pay for care for injuries resulting directly from being in the study. If you want information about those

circumstances or if you think you have suffered a research related injury let the study physicians know right away.

Will I be compensated for my participation?

If you agree to take part in this research study, we will pay you $40 plus parking for your time and effort after conclusion of the in-person data collection in Children's Rehab Center.

Payment will be made using a Target gift card after your completed visit. Payment you receive as compensation for participation in research is considered taxable income. If payment to an individual equals or exceeds $600 in any one calendar year, the University of Minnesota is required to report this information to the Internal Revenue Service (IRS). Research payments to study participants that equal or exceed $600 during any calendar year will result in a FORM 1099 (Miscellaneous Income) being issued to you and a copy sent to the IRS.

Optional Elements:

The following research activities are optional, meaning that you do not have to agree to them in order to participate in the research study. Please indicate your willingness to participate in these optional activities by placing your initials next to each activity.

Yes, I agree	**No, I disagree**	
_____	_____	The investigator may audio or video record me to aid with data analysis. The investigator will not share these recordings with anyone outside of the immediate study team.
_____	_____	The investigator may audio or video record me for use in scholarly presentations or publications. My identity may be shared as part of this activity.
_____	_____	The investigator may contact me in the future to see whether I am interested in participating in other research studies by Dr. Linda Koehler or Renata Braudy

Signature Block for Capable Adult:

Your signature documents your permission to take part in this research. You will be provided a copy of this signed document.

_____ _____

Signature of Participant Date

_____
Printed Name of Participant

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Signature Block for Witness:

WITNESS STATEMENT:
The participant was unable to read or sign this consent form because of the following reason:

☐ The participant is illiterate

☐ The participant is visually impaired

☐ The participant is physically unable to sign the consent form. Please describe:

_____

☐ Other *(please specify)*:

_____

APPENDIX H

Consent Form (includes HIPAA Authorization)

Title of Research Study*: Postoperative Radiotherapy Breast Cancer Treatment: Musculoskeletal and Functional Implications*
Investigator Team Contact Information: *Linda Koehler, PhD, PT*
For questions about research appointments, the research study, research results, or other concerns, call the study team at:

Investigator Name: Linda Koehler, PhD, PT Investigator Departmental Affiliation: Rehabilitation Medicine Phone Number: 612-626-1502 Email Address: koeh0139@umn.edu	Study Staff: Renata Braudy, PT, MS, MA, OCS, CLT, PhD Candidate Phone Number: na Email Address: rbraudy@umn.edu

Supported By: This research is supported by ***American Physical Therapy Association Promotion of Physical Therapy (PODS) I Scholarship and the Council of Graduate Studies at the University of Minnesota.***

Key Information About This Research Study

The following is a short summary to help you decide whether or not to be a part of this research study. More detailed information is listed later on in this form.

What is research?

Doctors and investigators are committed to your care and safety. There are important differences between research and treatment plans:

- The goal of research is to learn new things in order to help groups of people in the future. Investigators learn things by following the same plan with a number of participants, so they do not usually make changes to the plan for individual research participants. You, as an individual, may or may not be helped by volunteering for a research study.
- The goal of clinical care is to help you get better or to improve your quality of life. Doctors can make changes to your clinical care plan as needed.

Research and clinical care are often combined. One purpose of this informed consent document is to provide you clear information about the specific research activities of this study.

Why am I being asked to take part in this research study?

We are asking you to take part in this research study because you are a healthy female over 18 years of age without any history of shoulder problems.

What should I know about a research study?

- Someone will explain this research study to you.
- Whether or not you take part is up to you.
- You can choose not to take part.
- You can agree to take part and later change your mind.
- Your decision will not be held against you.
- You can ask all the questions you want before you decide.

Why is this research being done?

This research is part of a larger study that involves women with breast cancer. The overall purpose of this research is to better understand why some people have shoulder problems after surgery and radiation for breast cancer. Shoulder pain, stiffness, and/or weakness are common side effects of breast cancer treatment but are not well understood. This study will give us more information about why shoulder problems might occur, so that in the future we can identify and treat them better. The results of this study may help with the development of future rehabilitation programs designed to better identify and treat shoulder pain after breast cancer treatment.

How long will the research last?

We expect that you will be in this research study for approximately 1 hour after the informed consent is completed.

What will I need to do to participate?

You will need to come to the Cancer Survivorship and Lymphology Research Lab on the East Bank of the University of Minnesota for approximately 1 hour. We will perform an ultrasound of 3 chest wall muscles on both sides of your body.

More detailed information about the study procedures can be found under ***"What happens if I say yes, I want to be in this research?"***

Is there any way that being in this study could be bad for me?

There are no significant risks associated with this study, and the measurements are non-invasive. If you have shoulder pain when you move your arm, you can stop at any time. There is a small risk of loss of confidentiality, but this will be minimized by using secure data storage and by assigning each participant a non-identifiable study number that has approved password protection required for access.

You will be offered a separate authorization form that permits use of University of Minnesota Email ("Unsecured Email Correspondence") in place of encrypted emails for communication, ie scheduling. You do not have to sign the Unsecured Email Correspondence form in which case the study staff will communicate via encrypted email only.

More detailed information about the risks of this study can be found under ***the "What happens to the information collected for the research?" section***.

Will being in this study help me in any way?

We cannot promise any benefits to you or others from your taking part in this research. However, possible benefits include:

If you have shoulder pain and have not received medical care for it, we will discuss whether a referral to a physician or to physical therapy would be helpful to you. Additionally, some of this information may help guide physical therapy treatment for any shoulder pain or discomfort you may have. The information learned from this study can also help guide future medical treatment of shoulder problems after breast cancer treatment.

What happens if I do not want to be in this research?

There are no known alternatives, other than deciding not to participate in this research study.

Detailed Information About This Research Study

The following is more detailed information about this study in addition to the information listed above.

How many people will be studied?

We expect about 5 people here will be in this research study out of 5 people in the entire study nationally. This is a part of a larger study of 30 women with breast cancer by the same investigators.

What happens if I say "*Yes, I want to be in this research*"?

If you decide to participate in this research, after signing this informed consent and HIPPA form, you will be asked to come to the Cancer Survivorship and Lymphology Research Lab in the Children's Rehab Building on the East Bank of the University of Minnesota. Renata Braudy, PT(study staff) will meet you to perform the ultrasound.

Ultrasound will be used to 'see' 3 muscles in your chest wall on both the affected and unaffected sides. This information will tell us the size of those muscles as well as if and how much scar tissue is present.

All of the items listed above are for research purposes only and do not constitute standard care.

What happens if I say "Yes", but I change my mind later?

If you take part in this research study, and want to leave, you should tell us. Your choice not to be in this study will not negatively affect your right to any present or future medical care, your academic standing as a student, or your present or future employment.

We will make sure that you stop the study safely. We will also talk to you about follow-up care, if needed.

If you stop being in the research, information about you that has already been collected may not be removed from the study database. In that case, the data that we were able to collect may be used in the study analysis, but only as de-identified data.

Can I be removed from the research?

It's possible that we will have to ask you to leave the study before you finish it. If this happens, we will tell you why. We will also help arrange other care for you, if needed.

Will it cost me anything to participate in this research study?

Taking part in this research study will not lead to any costs to you.

Will being in this study help me in any way? (Detailed Benefits)

We cannot promise any benefits to you or others from your taking part in this research. However, your participation may help others in the future. For example, improved understanding of why some people have shoulder problems after breast cancer surgery and radiation can lead to improved identification and treatment of their shoulder problems.

What happens to the information collected for the research, including my health information?

We try to limit the use and sharing of your information, including research study records, any medical records and any other information about you, to people who have a need for this information. But we cannot promise complete confidentiality.

Overview

If you participate in this study, your information, including your health information, will be used and shared for purposes of conducting this research. As described later in this Consent Form, your information may also be used and shared for publishing and presenting the research results, future research, and any optional elements of the research you agree to in this Consent Form, which may include creating audio and video recordings of you. If you sign this Consent Form, you are giving us permission to use and share your health information for these purposes, and if we are using your medical records, you are giving permission to any health care providers who are treating you to share your medical records with us.

What health information will be made available?

Health information about you to be used and shared for the research includes those items checked by the research team below:

☒ Your medical records, which may include records from hospital and clinic visits, emergency room visits, immunizations, medical history and physical exams, medications, images and imaging reports, progress notes, psychological tests, EEG/EKG/ECHO reports, lab and pathology reports, dental records and/or financial records. These records may be used and shared for as long as this research continues.

☒ Information collected as part of this research study, including research procedures, research visits, and any optional elements of the research you agree to, all as described in this Consent Form. This information might not be part of your medical record, and may include things like responses to surveys and questionnaires, and information collected during research visits described in this Consent Form.

What about more sensitive health information?

Some health information is so sensitive that it requires your specific permission. If this research study requires any of this sensitive information, the boxes below will be marked and you will be asked to initial to permit this information to be made available to the research team to use and share as described in

this Consent Form.

☐ My drug & alcohol abuse, diagnosis & treatment records _____ (initial)

☐ My HIV/AIDS testing records _____ (initial)

☐ My genetic testing records _____ (initial)

☐ My mental health diagnosis/treatment records ____ (initial)

☐ My sickle cell anemia records _____ (initial)

Who will access and use my health information?

If you agree to participate in this study, your information will be shared with:

- The University of Minnesota research team and any institutions or individuals collaborating on the research with us;
- Others at the University of Minnesota and M Health/Fairview who provide support for the research or who oversee research (such as the Institutional Review Board or IRB which is the committee that provides ethical and regulatory oversight of research at the University, systems administrators and other technical and/or administrative support personnel, compliance and audit professionals (Such as the Quality Assurance Program of the Human Research Protection Program (HRPP)) , individuals involved in processing any compensation you may receive for your participation, and others);
- The research sponsor(s), any affiliates, partners or agents of the sponsor(s) involved in the research, organizations funding the research, and any affiliates, partners or agents of the funding organization(s) involved in the research;

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- Organizations who provide accreditation and oversight for research and the research team, and others authorized by law to review the quality and safety of the research (such as U.S. government agencies like the Food and Drug Administration, the Office of Human Research Protections, the Office of Research Integrity, or government agencies in other countries); and
- Organizations that process any payments that may be made to you for participating in this study, and any other individuals or organizations specifically identified in this Consent Form.

Additional sharing of your information for mandatory reporting

If we learn about any of the following, we may be required or permitted by law or policy to report this information to authorities:

- Current or ongoing child or vulnerable adult abuse or neglect;
- Communicable, infectious or other diseases required to be reported under Minnesota's Reportable Disease Rule;
- Certain wounds or conditions required to be reported under other state or federal law; or
- Excessive use of alcohol or use of controlled substances for non-medical reasons during pregnancy.

How will my information be used in publications and presentations?

We may publish the results of this research in scientific, medical, academic or other journals or reports, or present the results at conferences. Information that makes it easy to identify you (such as your name and contact information, SSN and medical records number) will not be part of any publication or presentation. If you have an extremely unique or rare condition that is not shared by many others, it is possible that some people may be able to determine your identity even without these identifiers.

What will be done with my data when this study is over?

We will use and may share data for future research. They may be shared with researchers/institutions outside of University of Minnesota. This could include for profit companies. We will not ask for your consent before using or sharing them. We will remove identifiers from your data and/or specimens, which means that nobody who works with them for future research will know who you are. Therefore, you will not receive any results or financial benefit from future research done on your specimens or data.

If you leave the study, you can ask to have the data collected about you removed. You can also ask us to remove information that identifies you from the data or samples. This may not be possible if your data has already been shared.
Please indicate whether you will allow the identifiable data to be used for future research by putting your initials next to one of the following choices:

_____ (initials) NO, my identifiable data may not be used for future research. They may be used for this study only.
_____ (initials) YES, my identifiable data may be used for other future research studies

Do I have to sign this Consent Form and give my permission to make my information, including my health information, available for use and sharing?

No, you do not have to sign this Consent Form. But if you do not sign, you will not be able to participate in this research study. Treatment available outside of the study, payment for such treatment, enrollment in health insurance plans and eligibility for benefits will not be impacted by your decision about signing this Consent Form.

Does my permission for making my health information available for use and sharing ever expire?

No, there is no expiration date.

May I cancel my permission for making my health information available for use and sharing?

Yes. You may cancel your permission at any time by writing to the researcher at the address on the first page of this Consent Form. If you cancel your permission, you will no longer be in the research study. You may also want to ask someone on the research team in canceling will affect any research related medical treatment. If you cancel your permission, any health information about you that was already used and shared may continue to be used and shared for the research study and any optional elements of the study to which you agree in this Consent Form.

What happens to my health information after it is shared with others?

When we share your information with others as described in this Consent Form, privacy laws may no longer protect your information and there may be further sharing of your information.

Will I be able to look at my records?

It is possible that the research team may not allow you to see the information collected for this study. However, you may access any information placed in your medical records after the study is complete.

Will I receive research test results?

Most tests done on samples in research studies are only for research and have no clear meaning for health care. If the research with your identifiable information or samples gives results that do have meaning for your health, the investigators will contact you to let you know what they have found.

Will anyone besides the study team be at my consent meeting?

You may be asked by the study team for your permission for an auditor to observe your consent meeting. Observing the consent meeting is one way that the University of Minnesota makes sure that your rights as a research participant are protected. The auditor is there to observe the consent meeting, which will be carried out by the people on the study team. The auditor will not document any personal (e.g. name, date of birth) or confidential information about you. The auditor will not observe your consent meeting without your permission ahead of time.

Whom do I contact if I have questions, concerns or feedback about my experience?

This research has been reviewed and approved by an IRB within the Human Research Protections Program (HRPP). To share feedback privately with the HRPP about your research experience, call the Research Participants' Advocate Line at 612-625-1650 (Toll Free: 1-888-224-8636) or go to z.umn.edu/participants. You are encouraged to contact the HRPP if:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You want to talk to someone besides the research team.
- You have questions about your rights as a research participant.

- You want to get information or provide input about this research.

Will I have a chance to provide feedback after the study is over?

The HRPP may ask you to complete a survey that asks about your experience as a research participant. You do not have to complete the survey if you do not want to. If you do choose to complete the survey, your responses will be anonymous.

If you are not asked to complete a survey, but you would like to share feedback, please contact the study team or the HRPP. See the "Investigator Contact Information" of this form for study team contact information and "Whom do I contact if I have questions, concerns or feedback about my experience?" of this form for HRPP contact information.

What happens if I am injured while participating in this research?

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to you or your insurance company. Under some circumstances the sponsor of the study will pay for care for injuries resulting directly from being in the study. If you want information about those circumstances or if you think you have suffered a research related injury let the study physicians know right away.

Will I be compensated for my participation?

If you agree to take part in this research study, we will pay you $20 plus parking for your time and effort after conclusion of the in-person data collection in Children's Rehab Center.

Payment will be made using a Target gift card after your completed visit. Payment you receive as compensation for participation in research is considered taxable income. If payment to an individual equals or exceeds $600 in any one calendar year, the University of Minnesota is required to report this information to the Internal Revenue Service (IRS). Research payments to study participants that equal or exceed $600 during any calendar year will result in a FORM 1099 (Miscellaneous Income) being issued to you and a copy sent to the IRS.

Optional Elements:

The following research activities are optional, meaning that you do not have to agree to them in order to participate in the research study. Please indicate your willingness to participate in these optional activities by placing your initials next to each activity.

Yes, I agree	**No, I disagree**	
_____	_____	The investigator may audio or video record me to aid with data analysis. The investigator will not share these recordings with anyone outside of the immediate study team.
_____	_____	The investigator may audio or video record me for use in scholarly

_____ _____ presentations or publications. My identity may be shared as part of this activity.

_____ _____ The investigator may contact me in the future to see whether I am interested in participating in other research studies by Dr. Linda Koehler or Renata Braudy

Signature Block for Capable Adult:

Your signature documents your permission to take part in this research. You will be provided a copy of this signed document.

_____ _____
Signature of Participant Date

_____
Printed Name of Participant

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

Signature Block for Witness:

WITNESS STATEMENT:
The participant was unable to read or sign this consent form because of the following reason:
☐ The participant is illiterate

☐ The participant is visually impaired

☐ The participant is physically unable to sign the consent form. Please describe:

_____

☐ Other *(please specify)*: _____

APPENDIX I

Table 33. Scapulothoracic posterior tilt (in degrees) on the affected and unaffected sides expressed as a function of hand dominance.

Humerothoracic Angle	**Cancer on dominant side,** n=12[1]		**Cancer on nondominant side,** n=14[1]		***p*-value[2]**
	Affected	**Unaffected**	**Affected**	**Unaffected**	
Rest (abduction and forward flexion)	-25 (16)	-22 (19)	-16 (14)	-23 (13)	0.3
30° abduction	-28 (14)	-26 (17)	-20 (14)	-27 (12)	0.3
60° abduction	-31 (14)	-29 (17)	-23 (14)	-31 (11)	0.2
90° abduction	-34 (13)	-32 (18)	-27 (15)	-35 (11)	0.2
120° abduction	-39 (14)	-37 (19_	-30 (16)	-40 (12)	0.3
30° forward flexion	-24 (13)	-22 (17)	-17 (14)	-23 (11)	0.4
60 ° forward flexion	-25 (13)	-23 (17)	-18 (14)	-24 (11)	0.4
90 ° forward flexion	-28 (13)	-24 (17)	-21 (15)	-27 (11)	0.2
120° forward flexion	-33 (14)	-30 (18)	-31 (15)	-27 (17)	0.3

[1] Mean (Standard deviation) in degrees

[2] Wilcoxon rank sum exact test comparing the difference of affected – unaffected of the dominant vs nondominant sides

Table 34. Sternoclavicular retraction (in degrees) on the affected and unaffected sides expressed as a function of hand dominance.

Humerothoracic Angle	**Cancer on dominant side,** n=12[1]		**Cancer on nondominant side,** n=14[1]		***p*-value[2]**
	Affected	**Unaffected**	**Affected**	**Unaffected**	
Rest (abduction and forward flexion)	-25 (13)	-22 (19)	-16 (14)	-23 (13)	0.3
30° abduction	-28 (14)	-26 (17)	-20 (14)	-27 (12)	0.3
60° abduction	-31 (14)	-29 (17)	-23 (14)	-31 (11)	0.2
90° abduction	-34 (13)	-32 (18)	-27 (15)	-35 (11)	0.2
120° abduction	-39 (14)	-37 (19)	-30 (16)	-40 (12)	0.3
30° forward flexion	-24 (12)	-22 (19)	-16 (14)	-23 (13)	0.4
60 ° forward flexion	-25 (13)	-23 (17)	-18 (14)	-24 (11)	0.4
90 ° forward flexion	-28 (13)	-24 (17)	-21 (15)	-27 (11)	0.2
120° forward flexion	-33 (14)	-30 (18)	-27 (17)	-34 (12)	0.3

[1] Mean (Standard deviation) in degrees
[2] Wilcoxon rank sum exact test comparing the difference of affected – unaffected of the dominant vs nondominant sides