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Protocol #: 21-5074 NCT06518174

Version Date: August 29, 2025

Project Title: A Novel Imaging Modality to Evaluate Radiation-Induced Uterine Injury

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I. Hypotheses and Specific Aims:

The goal of this pilot study is to assess the ability of a new imaging modality, shear-wave-elastography (SWE) ultrasound and magnetic resonance imaging (MRI) to identify radiation-induced uterine injury (RIUI). We will recruit female patients treated with pelvic radiation (PRT) at least 12 months prior to study initiation and before 40 years of age. We selected 12 months to increase the likelihood of occurrence of uterine fibrosis. We will perform transabdominal standard B-mode and Doppler US, SWE US, and pelvic MRI and characterize uterine parameters. For simplicity, the B-mode and Doppler US will be referred to collectively as US and shear-wave elastography ultrasound will be referred to as SWE. This study will provide essential information to design an intervention trial of a novel therapeutic approach to the treatment of radiation-induced uterine damage.

Aim 1: Assess the feasibility of SWE and pelvic MRI to identify and characterize uterine fibrosis. *Hypothesis: A grid map sampling of the uterus will indicate feasibility of SWE and pelvic MRI to detect and characterize uterine fibrosis*.

SWE and MRI will be performed at time of study recruitment. US procedures will be transabdominal. When possible, and with participant consent, transvaginal US will also be performed. MRI images will also be sampled from existing pretreatment studies performed as standard of care.

Perform SWE and MRI sampling of the uterus from fundus to lower uterine segment at two locations each from the upper, mid, and lower thirds of the uterus (six grid sample locations). Obtain five measurements at each location to evaluate for the presence and degree of fibrosis:

a. Assess the relationship between SWE measurements in kiloPascals (kPa) and each MRI parameter among cases at the post-treatment timepoint.
b. Assess the relationship between SWE measurements and each MRI parameter in kPa among controls at one random timepoint.
c. Evaluate differences in kPa between cases and controls according to SWE at the post-treatment timepoint.

The imaging study will be considered feasible if at least 85% (e.g., ≥15/17) of all enrolled cases achieve the diagnosability criteria by SWE, defined as having the interquartile range to median ratio (IQR/M) of the measurements (kPa) <=30% at any of the six locations.

Aim 2: Characterize uterine parameters after PRT.
Hypothesis: Patients treated with PRT will have decreased uterine volume and increased arterial resistive indices (blood flow) on US, and abnormal uterine parameters on MRI.

2a. Perform US and estimate uterine volumes and arterial resistance. Use descriptive statistics to compare values to controls in the same age group.

2b. Perform MRI and describe changes in each parameter among cases between the pre- and post-treatment timepoints. Assess changes in: size of the uterus from baseline, junctional zone anatomy, endometrial thickness, cervical length, myometrial thickness, T2 signal of the myometrium (normalized for skeletal muscle signal acting as an internal control), and enhancement features.

II. **Background and Significance**:
Radiation-induced uterine injury (RIUI) has far-reaching consequences on future fertility and pregnancy outcomes, leading to diminished quality of life for female cancer survivors and their children.[1-3] RIUI is associated with a 2- to 4-fold increased risk of infertility, perinatal loss, and premature birth.[1] Pelvic radiotherapy (PRT) and total body irradiation (TBI) are key causes of RIUI, characterized by fibrosis of the pelvic vasculature and myometrium.[2] This radiation-induced fibroatrophic (RIF) process presents as early, potentially reversible inflammation and late irreversible fibrosis.[4] Previous work in this area has focused on methods to identify and mitigate ovarian injury after cancer therapy,[5] but far less attention has been directed towards optimizing the uterine environment after radiation. Thus, even when ovarian function is protected, fertility and pregnancy outcomes remain significantly compromised after radiation injury to the uterus, presenting a critical gap in maternal-child health after cancer.[6]

Effective strategies to identify RIUI are urgently needed to protect female cancer survivors' reproductive health and the long-term health of their offspring.[7] Magnetic resonance imaging (MRI) is the gold standard to assess RIF of abdominopelvic organs;[8, 9] however, widespread use is cost prohibitive. Moreover, while MRI features of uterine fibrosis have been defined, they have not been widely validated. While qualitative assessment of various uterine morphologic and physiologic parameters before and after therapy can be used to determine fibrosis, there are no widely used defined thresholds to establish uterine fibrosis.[8-10] Previously defined morphologic parameters which can aid in assessment of fibrosis includes loss of junctional zone anatomy, cervical shortening, smaller uterine size, and decreased muscle thickness.[9, 10]

Shear-wave elastography (SWE) ultrasound (US) is an emerging low cost tool proven successful in identifying fibrosis of the liver and breast and in evaluating uterine leiomyomas and endometrial cancer.[11-19] SWE uses acoustic radiation force impulse (ARFI) to measure tissue stiffness, with results quantified and reported in kPa. It is in widespread use in quantifying liver fibrosis with existing reference range defined.[20] However, there are no studies using SWE to assess RIUI to correlate the degree of uterine damage and fertility outcomes, and no currently described reference standards.

III. Research Methods
As part of this pilot study, we aim to enroll a cohort of female patients who have undergone PRT. Through Health Data Compass, we have identified 17 female patients who received ≥ 50 Gy PRT > 12 months prior to study recruitment in the University of Colorado Health system (UCH) from January 2016 – December 2023. While we focus on patients who received higher

doses of radiation to the uterus, we will allow enrollment of patients who received any dose of PRT to facilitate recruitment.We anticipate enrolling up to 17 patients. While some of these patients may have been seen for their clinical care at any of the UCH sites, we will conduct the single study visit at the UCHealth main campus. We will also identify five female control subjects in the same age group, who have not received pelvic radiation, and who do not have pre-existing uterine lesions (such as fibroids). Enrolled subjects will have one study visit with the following data collected.

A. Outcome Measures:

1. SWE (post-treatment)
    i. SWE IQR/M
    ii. SWE Tissue Stiffness (kPa)
    iii. Uterine volume
    iv. Uterine arterial resistance blood flow
2. MRI (pre- and post- treatment)
    i. Size of uterus
    ii. Junctional zone anatomy
    iii. Endometrial thickness
    iv. Cervical length
    v. Myometrial thickness
    vi. T2 signal of the myometrium
    vii. Enhancement features

B. Description of Population to be Enrolled:

a. Cases
    i. Inclusion criteria:
        1. Female
        2. Less than 40 years old at the time of pelvic radiation
        3. ≥ 18 and < 45 years of age at time of enrollment
        4. Treated with pelvic radiation at UCHealth
        5. Intact uterus
        6. Received radiation to the uterus
        7. Pelvic MRI taken before the pelvic radiation
    ii. Exclusion criteria:
        1. History of hysterectomy
        2. Currently pregnant
        3. Known uterine malformation

b. Controls
    i. Inclusion criteria:
        1. Female
        2. ≥ 18 and < 45 years of age at time of enrollment
        3. No known history of pelvic radiation
        4. No known history of uterine fibroids or adenomyosis
        5. No uterine fibroids on pelvic ultrasound measuring > 2 cm
        6. No history of uterine surgery (hysteroscopic or abdominal myomectomy, cesarean section)
        7. No history of primary ovarian insufficiency/premature menopause
        8. No current or previous use of hormone therapy within 6 months
        9. Previous or current hormonal contraception (including LNG-IUD) is acceptable
    ii. Exclusion criteria:

1. Currently pregnant
2. Known uterine malformation

C. Study Design and Research Methods: This will be a pilot study.

The radiation oncologist (Co-I) will identify patients seen at the University of Colorado Health System (UCH) after January 1, 2016 who meet the study criteria. The research team will identify the controls during established visits in the Ob/Gyn clinic. A member of the study team will contact the patient to explain the study. If the patient would like to learn more about the study or participate, they will review the study in further detail via video conference or in-person and obtain consent/assent, if interested. Participants 18 and older will be consented. The study visit will be scheduled at that time. Study visit will be scheduled 1 week after menstrual cycle except in amenorrheic patients, for whom the study visit may be scheduled at any time.

We will obtain approval to use a short form as needed. If we use three short forms of any given language, we will translate the consent document.

The study visit includes:

1. Pregnancy test
2. Gynecologic and obstetric history questionnaire to be administered by the study coordinator via tablet into REDCap
3. Pelvic MRI without and with contrast (if patient consents to contrast)
4. Transabdominal pelvic ultrasound using standard B-mode and Doppler US and SWE
5. Optional transvaginal ultrasound using standard B-mode and Doppler US and SWE

Medical information will be abstracted from the electronic medical records (EMR) and include, but not be limited to:

a. Patient demographics
b. Cancer diagnosis, treatment, and associated ICD-10 codes
c. Imaging reports
d. Past medical history including other medical diagnoses and surgeries
e. Gynecologic and obstetric history such as potential confounders for uterine fibrosis:
    - Uterine fibroids
    - Adenomyosis
    - Previous hysteroscopic or abdominal myomectomy
    - Cesarean section
    - Invasive placental disorders (increta, accrete, percreta)
    - Endometrial ablation or resection
    - Dilation and curettage
    - Diagnostic or operative hysteroscopy for other indications
    - Hormone therapy, including contraception history

D. Description, Risks and Justification of Procedures and Data Collection Tools: Systematic processes and measures that will be employed to minimize patient risks include ensuring patient privacy and protection of Protected Health Information (PHI).

Study participants will be thoroughly informed of any possible risks and can decline study participation or to withdraw from the study at any time per standard IRB procedures.

The study team will maintain all research records electronically (REDCap) or in a locked filing cabinet in a secure office. If consent and source documents need to be transported from the clinic to the team offices in the Academic Office Building, the study team will ensure that the documents are securely transported and will not make additional stops between the two buildings.

We will keep private all research records that identify the participant to the extent allowed by law. The investigative team maintains the right to keep, preserve, use, and dispose of the findings of this investigation in accordance with IRB guidelines. Investigational records from this study will be maintained in a confidential manner; participant names will not be associated with any published results. Participant information and study information will be pulled from the participant's chart and compiled separately in the REDCap database.

Conventional MRI is rarely associated with any adverse effects. Most contraindications are relative precautions, which can be divided into five groups: implanted devices and foreign bodies, unstable patients, pregnancy, gadolinium contrast agents, and other. Pregnant patients will not be enrolled in this study. Prior to scanning, a thorough safety screen of each patient for any implanted, embedded, or attached devices or foreign objects is mandatory per standard radiology protocol. In addition, these patients will be screened for claustrophobia per standard radiology protocol. It is possible that during the research study, the research team may notice an unexpected finding. Should this occur, the finding will be reviewed by the study doctor(s) and discussed with the participant as necessary.

Gadolinium contrast agents can cause an adverse reaction such as rash, hives, itching, mild headache, and nausea. More serious but rare side effects such as gadolinium toxicity and nephrogenic systemic fibrosis are most often seen in patients with severe renal disease. We will not administer Gadolinium contrast agents to patients with a history of renal disease, defined as:

- Dialysis
- Kidney transplant
- Single kidney
- Kidney surgery
- History of known cancer involving the kidney(s)
- History of Chronic Kidney Disease or prior history of Acute Kidney Injury

Diagnostic ultrasound is a safe procedure that uses low-power sound waves. There are no known risks or contraindications to the procedure itself. Transvaginal ultrasounds can cause some discomfort when the transducer is inserted. As these study participants may be experiencing radiation vaginitis, use of transvaginal ultrasound is an optional procedure and is not required as part of the study.

Data Safety Monitoring Plan

The principal investigator will be responsible for the conduct of this study, overseeing participant safety, executing the data and safety monitoring (DSM) plan, and complying

with all reporting requirements to local and federal authorities. This oversight will be accomplished through additional oversight from the Data and Safety Monitoring Committee (DSMC) at the University of Colorado Cancer Center (CU Cancer Center). The DSMC is responsible for ensuring data quality and study participant safety for all trials at the CU Cancer Center. A summary of the DSMC's relevant activities is as follows:

- Conduct of internal audits
- May submit recommendations for corrective actions to the CU Cancer Center's Executive Committee

Study audits conducted by the DSMC will consist of a review of the regulatory documents, consent forms, and source data verification. Documentation of the audit conducted by the DSMC will then need to be submitted to the IRB of record at the time of the IRB's continuing review of this trial (if applicable per IRB guidelines).

E. Potential Scientific Problems: Limitations of this study is that it is a pilot, and we will have small numbers. To ensure that we meet recruitment/enrollment targets, we will recruit patients from our UCH affiliate sites. However, study visits will occur at the Anschutz campus. Another limitation is the lack of pre-treatment SWE data.

F. Data Analysis Plan: Data will be abstracted and stored on the secure REDCap server at the University of Colorado. Data will be exported into statistical software for descriptive statistics and analyses listed in below.

Aim	Data source
Aim 1: Assess the feasibility of SWE and pelvic MRI to identify and characterize uterine fibrosis.	Using SWE and MRI images performed after recruitment and existing pretreatment MRI. We will collect transabdominal SWE for all patients and transvaginal SWE when possible.
Aim 2: Characterize uterine parameters after PRT.	US images performed after recruitment to capture uterine volume and resistive indices. We will collect transabdominal US for all patients and transvaginal US when possible.

Descriptive statistics

Each endpoint will be descriptively characterized with summary statistics and corresponding figures. Summary statistics for continuous variables will include the mean, standard deviation, median, minimum, and maximum. Summary statistics for categorical variables will include the frequency and percentage of patients in each category. When applicable, summary statistics will also be stratified by treatment group (cases/controls) and time (pre-treatment/post-treatment). EHR medical information and radiation dose will be analogously described. Since this is a feasibility study, no formal statistical hypothesis will be tested. Regression models will be considered secondarily to generate hypotheses.

Aim1: Assess the feasibility of SWE and pelvic MRI to identify and characterize uterine fibrosis

SWE feasibility

SWE will be considered feasible if at least 85% (e.g., ≥15/17) of all patients achieve the diagnosability criteria, defined as having at least 1 of 6 locations with IQR/M ≤ 30%. The IQR/M and the number of patients who achieved diagnosability will be descriptively characterized.

Compare the degree of fibrosis according to SWE and MRI parameters across treatment groups

Since fibrosis thresholds have not been thoroughly established for SWE tissue stiffness nor MRI parameters, continuous SWE tissue stiffness (kPA) will be compared separately to each MRI parameter: uterus size, junctional anatomy, endometrial thickness, cervical length, myometrial thickness, T2 signal, and enhanced features. Graphic display will be used to assess potential association between SWE tissue stiffness and the MRI parameters, while comparing the cases to the controls. MRI parameters associated with SWE tissue stiffness will be further evaluated using a mixed effects linear regression model, where we will regress SWE tissue stiffness on the MRI parameters. The correlation among the grid samples from a subject will be evaluated using compound symmetric covariance structure. Correlation between SWE and each MRI parameter will be assessed separately following Hamlett, et al. (2004)[7] with mixed effects models. Inference regarding the correlations will be obtained using normal approximation. Radiation dose will be added to the most parsimonious model described above, if appropriate. Otherwise, stratified descriptive statistics will be used to summarize the association between radiation dose and SWE tissue stiffness and between radiation doses and the associated MRI parameters using Pearson, or Spearman correlation, whichever is appropriate. Generalized mixed effects models will be used to assess the association between the categorical outcome presence of uterine myometrial fibrosis with the MRI parameters as described above. Violin or boxplots with overlayed scatterplots will be used to depict differences in SWE tissue stiffness across treatment groups.

Evaluate the correlation between MRI images collected at pretreatment and after recruitment, using repeated measures correlation.

Descriptive statistics and previously described figures will be similarly used to characterize the change in each MRI parameter between the pre-treatment and post-treatment timepoints. Additionally, linear mixed effects regression models will be performed separately with each MRI parameter. The predictors will include pre- and post- treatment time points and treatment group. If appropriate, radiation dose will be included in the model as well, where the radiation dose will be assigned as 0 for the control patients. Contrast statements will be used to assess the change in each outcome comparing treatment groups and the radiation doses effect.

Aim2: Characterize uterine parameters after PRT

On-study uterine volume and arterial resistance will then be assessed with SWE. Previously described descriptive statistics and plots will be used to depict differences in each measurement across cases and controls. Sample size permitting, t-tests will be considered to secondarily

quantify the differences across groups. Transabdominal and transvaginal SWE measurements will also be compared as an exploratory endpoint.

G. Summarize Knowledge to be Gained: Findings from this study may have the potential to identify a cost-effective imaging modality to identify RIUI. The pilot data will allow us to perform a larger R21 study to inform radiation dose cutoffs for RIUI and ultimately contribute to the development of a screening tool to identify women at greatest risk of adverse reproductive outcomes after radiation. Findings from this study will generate pilot data for an R21 submission to assess feasibility of SWE and MRI to identify uterine fibrosis. This knowledge may allow SWE to be used as a cost-effective method of identifying uterine injury compared to MRI.

H. References:

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