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# Cardiac Magnetic Resonance Imaging and Blood Biomarkers for Evaluation of Radiation-Induced Cardiotoxicity in Patients With Breast Cancer: Results of a Phase 2 Clinical Trial

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Clinical Investigation

# Cardiac Magnetic Resonance Imaging and Blood Biomarkers for Evaluation of Radiation-Induced Cardiotoxicity in Patients With Breast Cancer: Results of a Phase 2 Clinical Trial

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**Purpose:** Radiation therapy (RT) can increase the risk of cardiac events in patients with breast cancer (BC), but biomarkers predicting risk for developing RT-induced cardiac disease are currently lacking. We report results from a prospective clinical

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All data generated and analyzed during this study are included in this published article (and its supplementary information files).

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trial evaluating early magnetic resonance imaging (MRI) and serum biomarker changes as predictors of cardiac injury and risk of subsequent cardiac events after RT for left-sided disease.

**Methods:** Women with node-negative and node-positive (N-/+) left-sided BC were enrolled on 2 institutional review board (IRB)−approved protocols at 2 institutions. MRI was conducted pretreatment (within 1 week of starting radiation), at the end of treatment (last day of treatment ±1 week), and 3 months after the last day of treatment (±2 weeks) to quantify left and right ventricular volumes and function, myocardial fibrosis, and edema. Perfusion changes during regadenoson stress perfusion were also assessed on a subset of patients (n = 28). Serum was collected at the same time points. Whole heart and cardiac substructures were contoured using CT and MRI. Models were constructed using baseline cardiac and clinical risk factors. Associations between MRI-measured changes and dose were evaluated.

**Results:** Among 51 women enrolled, mean heart dose ranged from 0.80 to 4.7 Gy and mean left ventricular (LV) dose from 1.1 to 8.2 Gy, with mean heart dose 2.0 Gy. T1 time, a marker of fibrosis, and right ventricular (RV) ejection fraction (EF) significantly changed with treatment; these were not dose dependent. T2 (marker of edema) and LV EF did not significantly change. No risk factors were associated with baseline global perfusion. Prior receipt of doxorubicin was marginally associated with decreased myocardial perfusion after RT ($P$ = .059), and mean MHD was not associated with perfusion changes. A significant correlation between baseline IL-6 and mean heart dose (MHD) at the end of RT ($\rho$ 0.44, $P$ = .007) and a strong trend between troponin I and MHD at 3 months post-treatment ($\rho$ 0.33, $P$ = .07) were observed. No other significant correlations were identified.

**Conclusions:** In this prospective study of women with left-sided breast cancer treated with contemporary treatment planning, cardiac radiation doses were very low relative to historical doses reported by Darby et al. Although we observed significant changes in T1 and RV EF shortly after RT, these changes were not correlated with whole heart or substructure doses. Serum biomarker analysis of cardiac injury demonstrates an interesting trend between markers and MHD that warrants further investigation. Published by Elsevier Inc.

## Introduction

Based on multiple randomized trials and the subsequent Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis, significantly reduced rates of locoregional recurrence with adjuvant radiation occur after breast-conserving surgery and survival is significantly improved.[1] As more effective screening, surgical techniques, and systemic therapies are developed, there is increased interest in balancing the need for radiation treatment with its potential acute and late side effects. Although in women with breast cancer with regional nodal involvement, the addition of regional nodal radiation has been shown to improve disease-free survival and breast cancer −specific survival, this improved disease control comes at the cost of increasing dose to the heart, lung, and draining lymphatics.[2,3] Regional radiation therapy (RT) has been shown to increase rates of lymphedema and pneumonitis.[2,3] Similarly, radiation's late effects on the heart have been well documented in several retrospective cohort and observational trials,[4-10] but prospective evidence quantifying the damage and informing our understanding of its mechanisms remains lacking. From long-term follow-up of patients with lymphoma, it is known that radiation therapy can lead to an increased risk of myocardial infarction, valvular dysfunction, systolic and diastolic function abnormalities, and heart failure among cancer survivors.[11,12] Patients with breast cancer receive lower doses to smaller volumes of the heart compared with lymphoma patients, but with their excellent long-term survival, it is crucial to understand the potential effects of low-dose radiation therapy.

Although cardiac injury after breast radiation therapy remains a significant concern, there is a paucity of validated biomarkers of cardiac injury that either identify women at risk for cardiac injury or who have experienced clinically meaningful damage. Efforts to minimize heart dose delivered during breast radiation therapy have centered around several planning and treatment delivery techniques. These include patient positioning techniques such as treatment in the prone position; treatment planning techniques such as cardiac blocking, partial breast radiation, or intensity modulation; and treatment machine techniques that include respiratory gating, deep inspiration breath hold, and use of intraoperative electron radiation or protons instead of photons,[13,14] although some of these technologies are not yet widely available. Although effective, these strategies can still result in doses of radiation to be delivered to the heart with the potential for long-term negative effects. An increased understanding of cardiac injury susceptibility and need for these techniques represents an unfilled clinical need.

Cardiac MRI can be implemented to evaluate cardiac dysfunction including alterations in chamber structure and function, myocardial perfusion, and tissue properties using T1 and T2 mapping.[15,16] For detection of coronary artery disease, MRI outperforms single-photon emission computed tomography (SPECT) dobutamine stress echocardiography for the detection of ischemia, and features such as left-ventricular ejection fraction and inducible perfusion defects are predictive of major adverse cardiovascular events.[17-20] MRI also can detect wall-motion abnormalities, valvular dysfunction, inflammation, and myocardial fibrosis and edema.[21] Dynamic contrast-enhanced MRI (DCE-

MRI) during stress and rest can evaluate the combined effects of both microvascular and epicardial coronary artery disease.[22] Thus, cardiac MRI holds promise for early detection of subclinical cardiac abnormalities after radiation therapy and may potentially identify patients for aggressive intervention to prevent future cardiac events.

At present, various data exist on potential biomarkers of cardiac radiation exposure and damage, and these have not always been systematically assessed in a longitudinal fashion in patients undergoing breast radiotherapy.[23-28] The potential use of imaging and blood-based biomarkers suggests that additional study in this area is warranted. Potential biomarker candidates have been identified based on other processes affecting heart function in a way similar to probable mechanisms of RT-related injury and may be of use as indicators of early cardiac injury in women undergoing breast radiation therapy. For fibrosis and left ventricular dysfunction, these include galectin-3 and NT-Pro brain natriuretic peptide.[29,30] Other possibilities include for myocyte destruction, troponin, and for inflammation and oxidative stress, C-reactive protein, myeloperoxidase, and growth differentiation factor.[31]

To determine whether blood-based or imaging biomarkers are viable for the detection of early cardiac injury, we designed a prospective, single-arm, multi-institutional observational clinical study to assess early imaging with MRI and blood-based biomarkers of cardiac injury. We hypothesized that imaging and blood biomarkers of cardiac exposure might be used as a first step to identify patients at increased risk for cardiac effects. These patients could then be targeted for close monitoring and early intervention, potentially with medications such as statins or angiotensin-converting enzyme (ACE) inhibitors. Additionally, by characterizing a time-course and radiation dose-volume relationship, potentially real-time modifications might be made to RT field design for patients sensitive to RT effects. Identifying candidates could also enable prioritization of cardiac-sparing treatment planning and delivery techniques for future patients.

## Materials and Methods

### Clinical study

Two institutional review board (IRB)−approved protocols for single-arm clinical studies were developed to identify blood and imaging biomarker changes with cardiac exposure to radiation. The trial was registered with the National Institutes of Health national trials registry at clinicaltrials.gov with the trial IDs of NCT02494453 and NCT02496260. Patients who were to receive RT for left-sided breast cancer and who could undergo contrast-enhanced MRIs were eligible. Patients were recruited at both institutions between July 2015 and March 2018, and 56 patients were accrued with 5 screening ineligible. Figure 1 summarizes the study timeline with all evaluated patients undergoing 3 MRIs (before RT, immediately post-RT, and 3 months post-RT) and blood draws at corresponding timepoints.

### Radiation therapy

Conventional dose and fractionation was used for all patients (1.8-2.0 Gy/fraction) with total dose to 50 Gy with an optional boost allowed to 60 Gy in 2 Gy/fraction. No matched electron fields were allowed. All treatment was delivered with 3D conformal treatment planning (3DCRT) or intensity modulated radiation therapy (IMRT). Only photons were used for treatment with no use of protons.

### MRI

Serial ECG-gated steady-state free precession MRI with a body coil was performed to quantify left and right ventricular volumes and function, precontrast quantitative T1- and T2-mapping to myocardial fibrosis and edema, and regadenoson stress perfusion. Motion correction for T1, T2, and dynamic perfusion imaging was performed inline during image reconstruction.[32] Baseline MRI was performed after the completion of all chemotherapy. MRI imaging was performed using a standardized protocol on a designated Siemens 3T wide bore MRI scanner (Siemens Skyra, Siemens Medical Imaging, Erlangen, Germany) or a Philips 3T (Philips Ingenia, Philips Medical Systems, Cleveland, OH) wide bore MRI scanner. After image localization, ECG-gated steady-state free precession images were obtained in short-axis, 2-chamber, 3-chamber, and 4-chamber views. T1 and T2 mapping were performed before the administration of contrast. Dynamic contrast enhancement imaging was performed during infusion of gadopentetate dimeglumine at rest and for a subset of patients imaged on the Siemens scanner, during regadenoson stress (0.4 mg). A dose of 75 mg of aminophylline was administered intravenously

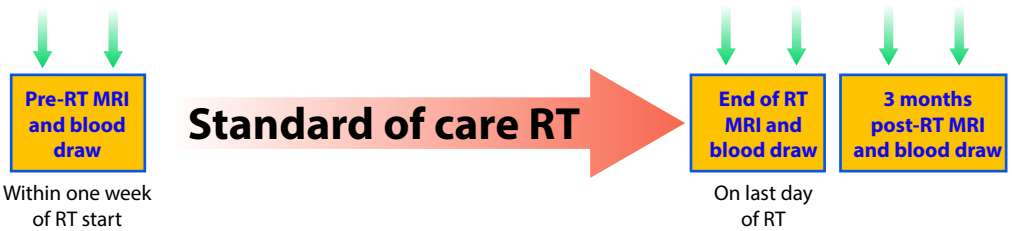


**Fig. 1.** Overview of the clinical study. *Abbreviations:* MRI = magnetic resonance imaging; RT = radiation therapy.

after completion of stress perfusion imaging to reverse the effects of regadenoson. Axial phase contrast imaging at the level of the left pulmonary artery and double oblique long-axis steady-state free precession imaging of the aorta were performed for quantification of aortic pulse wave velocity. LV and RV volumes and ejection fraction were quantified by manually tracing motion corrected end-diastolic and end-systolic frames on short-axis images. T1 and T2 were quantified on motion-corrected short-axis images acquired at the basal, midventricular, and apical levels of the left ventricle. Circumferential strain was quantified from ECG-gated short-axis images using speckle tracking.[33] Strain was quantified across all short-axis steady-state free precession slices and regional values computed globally and for regions of the left ventricle based on standard segmentation. Perfusion imaging was performed on 3 short-axis slices covering the base, mid, and apical left ventricle. Myocardial perfusion reserve index (MPRI) was computed globally and for regions of the left ventricle using standard segmentation. All MRI quantification was performed using a standard commercial digital imaging software tool for cardiac MRI (CMR42, Circle Cardiovascular Imaging, Calgary, Canada). Owing to known challenges with combining multivendor quantitative data, serial images were normalized such that subjects served as their own controls and the study site (ie, MRI vendor) was considered in subsequent analysis. MRI parameters assessed included myocardial precontrast T1, myocardial T2, left ventricular end-diastolic volume, left ventricular end-systolic volume, left ventricular ejection fraction, left ventricular mass, right ventricular end-diastolic volume, right ventricular end-systolic volume, right ventricular ejection fraction, myocardial perfusion reserve index (global), myocardial perfusion reserve index (anterior), left ventricular circumferential strain, quantitative T1 and T2 mapping, aortic pulse wave velocity, and, in a subset, stress myocardial perfusion reserve (Supplemental Fig. E1).

## Blood biomarkers

The following serum markers (high-sensitivity cardiac troponin I [cTnI; Singulex Erenna Immunoassay], endothelin 1 [ET-1; Singulex ELISA], interleukin 6 [IL-6; Singulex ELISA], high-sensitivity C-reactive protein [hsCRP; Singulex ELISA], cholesterol, high-density lipoprotein [HDL], low-density lipoprotein [LDL], triglycerides [Trig], and N-terminal pro B-type natriuretic peptide [NT pro-BNP; Singulex ELISA]) were collected for evaluation. Blood samples were collected at the same time points (pretreatment baseline, end of radiation treatment, and 3 months postradiation treatment) for all patients.

## Heart dose and contouring

The whole heart and cardiac substructures were contoured on the CT scan using a coregistered cardiac MR to supplement the information. Guidance of heart contouring was added by published heart atlases as well as the RTOG Atlas for Organs at Risk for Thoracic RT.[34,35] All contours were reviewed by radiation oncology attendings and edited for consistency. Cardiac substructures were contoured using MRI registered to CT scans and dose to the whole heart and cardiac substructures was calculated (Supplemental Fig. E2). For each patient, a rigid registration was performed for the T2 MR data sets to the CT simulation data set to enable contouring of substructures. The initial registration focused on the boundaries between the heart and other tissues. Then, the registration was biased toward the left ventricle region to account for proximity to the dose distribution and the regions of interest for the dosimetric evaluation. This methodology is consistent with that in American Association of Physicists in Medicine Task Group 132.[36] Scan segmentation was performed initially by a single trained radiation therapist with all tracings individually reviewed and adjusted by a single core cardiovascular training statement level 3−trained cardiologist with 13 years of cardiac MRI experience. All processing was consistent with Society for Cardiovascular Magnetic Resonance recommendations.[37] The following dose metrics were extracted and evaluated: whole heart maximum, mean, V50 Gy, V30 Gy, and V5 Gy, and total left ventricle (LV), anterior LV, inferior LV, lateral LV, and septal LV were extracted and evaluated for associations with MRI endpoints.

## Statistical analysis

Univariable regression models were fit to assess for correlation of baseline clinical factors and baseline imaging biomarkers. Longitudinal regression models were fit to test whether mean values of imaging biomarkers changed from baseline to end of treatment or 3-month timepoint. The impact of RT heart dose on change from baseline in imaging biomarkers was assessed using longitudinal regression models including patient level random intercepts to account for within patient between time correlation. These models also adjusted for doxorubicin by including it as a covariate. Associations between blood biomarkers and radiation dose were assessed using Pearson correlation coefficients with separate estimates obtained at each timepoint (baseline, post-treatment, or 3-month). The $P$ values shown in tables are nominal $P$ values, but the Benjamini-Hochberg method was implemented to address multiple testing between biomarkers and mean heart dose to control the false discovery rate (FDR) at 0.10. No FDR correction was used when assessing correlation among biomarkers. No imputation was performed for missing data. Statistical significance was defined as a 2-sided $P$ value $<.05$, although given the early stage of this research, we also note potentially interesting findings with $P$ values $<.10$. All analysis was performed using the R statistical package.

**Table 1** Demographics of the patients included in the trials (N = 51 patients)

| Patient characteristics | Mean (range) |
|---|---|
| Age | 56.8 (28-74) |
| Body mass index | 32.0 (20.3-48.7) |
| | **Number (percentage)** |
| Underlying coronary artery disease | 4/51 (8%) |
| Underlying cardiac risk factors* | 24/51 (47%) |
| Smoking (current) | 17/51 (33%) |
| History of adriamycin | 13/51 (25%) |
| African American | 24/51 (47%) |
| Caucasian | 26/51 (51%) |
| Asian | 1/51 (2%) |

* Underlying cardiac risk factors (hypertension, hyperlipidemia, or type 2 diabetes).

## Results

### Patient characteristics

Between and June 2015 and July 2017, 51 women with left-sided breast cancer from 2 institutions were enrolled with clinical and relevant cardiac-related demographic information summarized in Table 1. As anthracycline receipt was part of the chemotherapy regimen for almost all women receiving chemotherapy (12/13 women), anthracycline receipt was listed as representative in the table. Similarly, as only 1 woman received anti-HER2 therapy, this is not included in the table. As part of the study, pretreatment blood draws and MRI before the initiation of treatment were performed, as well as immediately at the end of the radiation therapy course of treatment and again 3 months after treatment completion (Fig. 1).

### Heart dose

In this cohort, the mean heart dose (MHD) for all patients was 2.0 Gy with a range of 0.80 to 4.69 Gy (Supplemental Fig. E3). The mean LV dose was 3.0 Gy with a range of LV 1.10 to 8.2 Gy (Supplemental Table E1). The average whole heart maximum dose (to 0.1 $cm^3$) ranged from 7.2 to 58.6 Gy with an average maximum dose of 29.8 Gy. Additional heart dose metrics used in the analysis (V50, V30, V5, and anterior, inferior, lateral, and septal LV doses) are shown in Supplemental Table E1.

### Clinical factors and influence on MRI features at baseline

The analysis of associations among baseline clinical factors (age, underlying coronary artery disease [CAD], cardiac risk, smoking, body mass index [BMI], and doxorubicin) and MRI metrics performed at baseline, end of treatment, and 3 months post-treatment did not reveal any significant associations between clinical factors and MRI changes (Supplemental Table E2). Additional analysis in which patient and clinical characteristics of age, cardiac risk, underlying CAD, smoking status, BMI, use of doxorubicin, and treatment site were modeled for associations with baseline measures of T1, right ventricular ejection fraction (RVEF), global myocardial perfusion reserve index (MPRI), or MPRI on the anterior wall also demonstrated no significant associations, although there was a trend for baseline MPRI anterior wall measurements ($P = .07$) with BMI that might become significant in a larger study.

### MRI changes after treatment

Example MRI parameters assessed included left and right ventricular volumes and ejection fractions, left ventricular circumferential strain, quantitative T1 and T2 mapping, aortic pulse wave velocity, and, in a subset of patients, stress myocardial perfusion reserve as shown in Supplemental Figure E1. On average a decrease in the T1 measure post-treatment compared with baseline was observed at both post-treatment scan time points (immediately at the end of treatment, −20, $P = .022$ and at 3 months, −23, $P < .001$). A decrease in the apical T1 signal at both the end of treatment (−22.29, $P = .008$) and the 3-month time point (−16.09, $P = .04$) was also observed. In addition, a significant decrease in RVEF was seen at the end of treatment (−4.63, $P = .003$); it was, however, no longer significant at the 3-month time point (−1.02, $P = .35$). On average, all other MRI endpoints (T2, LVEF, MPRI, and MPRI anterior wall) did not significantly change between baseline and post-treatment (Supplemental Table E3).

Associations among dose and age, CAD, cardiac risk, smoking, BMI, and doxorubicin receipt (strong trend in the baseline modeling) were then evaluated with changes in MRI measurements (T1, RVEF, MPRI, and MPRI anterior wall) (Supplemental Table E4). In this case, dose was considered either to the whole heart (as measured by mean heart dose) or to the left ventricle (as measured by mean dose to the left ventricle). Across all models there was no significant dose effect seen with any of the MRI measurements. This was true for dose defined as either mean dose to the entire heart or dose to the left ventricle. This was also true when considering whole heart maximum, mean, V50 Gy, V30 Gy, and V5 Gy, and total LV, anterior LV, inferior LV, lateral LV, and septal LV doses as there were no associations with MRI changes. The only variable that showed a strong trend with the MRI measurements across these MRI measurements was receipt of doxorubicin with outcome of change in MPRI (decreased perfusion, $P = .06$). Mean heart dose and mean left ventricle doses had no significant dose effect seen with any of the MRI measurements across all models tested (Supplemental Table E4).

**Table 2** Baseline biomarkers

| | n | Mean | SD | Median | Minimum | Maximum |
|---|---|---|---|---|---|---|
| cTnI, pg/mL | 41 | 3.13 | 4.57 | 1.00 | 0.20 | 18.10 |
| ET-1, pg/mL | 41 | 2.39 | 0.95 | 2.10 | 1.40 | 5.20 |
| IL-6, pg/mL | 41 | 2.66 | 2.37 | 1.90 | 0.20 | 10.80 |
| hsCRP, mg/L | 38 | 4.42 | 3.97 | 3.06 | 0.15 | 14.90 |
| CHOL, mg/dL | 38 | 200.26 | 52.42 | 196.50 | 73.00 | 395.00 |
| HDL mg/dL | 38 | 51.26 | 17.72 | 51.00 | 24.00 | 91.00 |
| TRIG, mg/dL | 38 | 136.53 | 71.85 | 121.00 | 60.00 | 414.00 |
| NT pro-BNP, pg/mL | 38 | 98.38 | 112.86 | 63.70 | 8.13 | 539.50 |
| LDL, mg/dL | 38 | 124.55 | 49.41 | 119.00 | 27.00 | 315.00 |

Biomarkers below the lower limit of detection (LLD) are included as LLD/2.

*Abbreviations:* CHOL = cholesterol; cTnI = cardiac troponin I; ET-1 = endothelin 1; HDL = high-density lipoprotein; hsCRP = high-sensitivity C-reactive protein; IL-6 = interleukin-6; LDL = low-density lipoprotein; NT pro-BNP = N-terminal pro B-type natriuretic peptide; TRIG = triglycerides.

**Table 3** Pearson correlation coefficients $\rho$ for baseline biomarkers

| Biomarker 1 | Biomarker 2 | $\rho$ | *P* value |
|---|---|---|---|
| CHOL, mg/dL | LDL, mg/dL | 0.95 | <.001 |
| ET-1, pg/mL | hsCRP, mg/L | 0.41 | .011 |
| ET-1, pg/mL | NT pro-BNP, pg/mL | 0.42 | .008 |
| ET-1, pg/mL | TRIG, mg/dL | 0.44 | .005 |
| HDL, mg/dL | TRIG, mg/dL | −0.49 | .002 |
| hsCRP, mg/L | HDL, mg/dL | −0.50 | .001 |
| IL-6, pg/mL | HDL, mg/dL | −0.32 | .048 |
| IL-6, pg/mL | hsCRP, mg/L | 0.45 | .005 |
| IL-6, pg/mL | NT pro-BNP, pg/mL | 0.45 | .004 |

*Abbreviations:* CHOL = cholesterol; ET-1 = endothelin 1; HDL = high-density lipoprotein; hsCRP = high-sensitivity C-reactive protein; IL-6 = interleukin-6; LDL = low-density lipoprotein; NT pro-BNP = N-terminal pro B-type natriuretic peptide; TRIG = triglycerides.

Only pairs with significant correlation ($P < .05$) are shown.

## Blood biomarkers of cardiac injury

Table 2 presents baseline levels of cTnl, ET-1, IL-6, hsCRP, cholesterol, HDL, LDL, Trig, and NT pro-BNP. There was a significant correlation among baseline levels of several of the biomarkers including cholesterol and LDL, ET-1, and hsCRP, NT pro-BNP and triglycerides, HDL and triglycerides, hsCRP and HDL, and IL-6 and HDL, hsCRP, and NT pro-BNP, as noted in Table 3. This significance persisted for multiple biomarkers at the end of treatment and at the 3-month post-treatment time points (Supplemental Tables E5, E6). At the completion of treatment, a significant positive correlation existed between MHD and IL-6 levels ($\rho$ 0.44, $P$ = .007) but not with any of the other serum-based markers (Table 4). Of note, this correlation between MHD and IL-6 levels was not maintained at the 3-month time point. Likewise, at 3 months post-treatment, a strong trend existed between mean heart dose and cardiac troponin I (cTnI) ($\rho$ 0.33, $P$ = .07) but none of the other serum markers (Table 5). There was a strong, although not significant, positive trend between mean heart dose and

**Table 4** Pearson correlation coefficients $\rho$ between biomarkers (post-treatment absolute value) and mean heart dose

| Biomarker | Mean heart dose | $\rho$ | *P* value |
|---|---|---|---|
| CHOL, mg/dL | Mean heart dose | −0.18 | .285 |
| cTnI, pg/mL | Mean heart dose | 0.27 | .106 |
| ET-1, pg/mL | Mean heart dose | 0.17 | .310 |
| HDL, mg/dL | Mean heart dose | −0.10 | .548 |
| hsCRP, mg/L | Mean heart dose | −0.02 | .889 |
| **IL-6, pg/mL** | **Mean heart dose** | **0.44** | **.007*** |
| LDL, mg/dL | Mean heart dose | −0.22 | .196 |
| NT pro-BNP, pg/mL | Mean heart dose | 0.26 | .122 |
| TRIG, mg/dL | Mean heart dose | 0.11 | .515 |

*Abbreviations:* CHOL = cholesterol; cTnI = cardiac troponin I; ET-1 = endothelin 1; HDL = high-density lipoprotein; hsCRP = high-sensitivity C-reactive protein; IL-6 = interleukin-6; LDL = low-density lipoprotein; NT pro-BNP = N-terminal pro B-type natriuretic peptide; TRIG = triglycerides.

Boldface indicates *P* value < .05

* Indicates significance at a false discovery rate = 0.10.

**Table 5** Pearson correlation coefficients $\rho$ between biomarkers (3-month absolute value) and mean heart dose

| Biomarker | Mean heart dose | $\rho$ | *P* value |
|---|---|---|---|
| CHOL, mg/dL | Mean heart dose | 0.23 | .251 |
| **cTnI, pg/mL** | **Mean heart dose** | **0.33** | **.073*** |
| ET-1, pg/mL | Mean heart dose | −0.29 | .110 |
| HDL, mg/dL | Mean heart dose | 0.27 | .166 |
| hsCRP, mg/L | Mean heart dose | −0.02 | .923 |
| IL-6, pg/mL | Mean heart dose | −0.04 | .835 |
| LDL, mg/dL | Mean heart dose | 0.20 | .320 |
| NT pro-BNP, pg/mL | Mean heart dose | −0.07 | .734 |
| TRIG, mg/dL | Mean heart dose | −0.20 | .322 |

*Abbreviations:* CHOL = cholesterol; cTnI = cardiac troponin I; ET-1 = endothelin 1; HDL = high-density lipoprotein; hsCRP = high-sensitivity C-reactive protein; IL-6 = interleukin-6; LDL = low-density lipoprotein; NT pro-BNP = N-terminal pro B-type natriuretic peptide; TRIG = triglycerides.

Boldface indicates *P* value < .05

* Indicates significance at a false discovery rate = 0.10.

markers of cardiac injury at the end of radiation treatment, specifically cTnI and NT pro-BNP (Table 4). Similarly, at 3 months postradiation, there was an inverse correlation between mean heart dose and ET-1, in addition to the strong trend toward positive correlation of MHD and cTnI at that time point (Table 5). Correlation matrices and scatter plots for biomarkers at baseline, at 3 months, and the change between them are included in Supplemental Figures E4 to E6.

## Discussion

In this prospective multi-institutional study of 51 patients with left-sided breast cancer undergoing radiation therapy, we found that heart doses were very low relative to historical doses reported by Darby et al, with a mean heart dose of 2.0 Gy. Assessment of early MRI changes after radiation therapy demonstrated that T1 time, a marker of fibrosis, shortened significantly and RV ejection fraction decreased significantly after treatment, but these changes were not dose dependent. T2 mapping, a marker of edema, and LVEF did not significantly change. No risk factors were associated with baseline global perfusion, although lower baseline anterior perfusion trended with higher baseline BMI ($P$ = .07). Additionally, prior receipt of doxorubicin was borderline significantly associated with changes in cardiac perfusion after RT (decreased perfusion, $P$ = .06), and MHD was not associated with perfusion changes. IL-6 levels significantly increased as a function of MHD immediately at the end of treatment, and there was a persistent positive correlation that trended toward significance for cardiac troponin I both at the end of treatment and at the 3-month time point. These data suggest that modern conventional MRI analysis tools and serum biomarkers may not be sufficiently sensitive to identify significant changes to the heart or circulating biomarkers and suggest that such injury may not occur with these low doses of radiation during the timeframe studied, though long-term cardiac outcomes are not yet available from this cohort.

This study builds on an extensive body of literature evaluating various imaging techniques to detect cardiac injury after breast radiation. Heggemann et al recently reported largely similar findings in a 49-patient prospective cohort study in which patients treated with 3-dimensional conformal radiation therapy (3DCRT) or IMRT showed transient decreases in EF with reduction in mitral and tricuspid annular plane systolic excursion, though this reduction was still within the normal range.[38] The authors concluded that at least within 24 months of breast radiation therapy, only subclinical cardiac changes were observed in patients treated with 3DCRT or IMRT. Likewise, Bergom et al recently noted that in 20 patients with node-positive breast cancer treated with anthracycline-based chemotherapy and 3DCRT, cardiac magnetic resonance (CMR) had no clinically abnormal CMR findings at a median follow-up time of 8.3 years.[39] Finally, Clasen et al recently found in a prospective longitudinal study of 86 patients with breast cancer treated with photon or proton radiation therapy that there are modest subclinical changes in measures of cardiac function in the short-term (LVEF and longitudinal strain) with no changes in circumferential strain or diastolic function.[40] In keeping with the results presented here, these and other studies suggest that current conventional imaging-based approaches may not be sensitive enough to detect clinically relevant cardiac dysfunction when cardiac radiation doses are kept low with modern treatment planning.

Interestingly, there was a trend between cardiac troponin 1 at end of treatment and at 3 months' post-treatment with the mean heart dose, although this did not reach statistical significance. This possibly suggests that myocardial injury may be occurring even in range of the very low heart doses in this study. A statistically significant relationship was noted between mean heart dose and IL-6, a marker of acute inflammation. This cytokine has complex effects in chronic and acute exposures that range from protective to proatherogenic.[41] These significant correlations even in a small number of patients with low doses of radiation to the heart in the present study suggest that further evaluation of these markers is warranted. IL-6 is a marker of inflammation that can be elevated in a variety of cardiac conditions including after radiation treatment.[42,43] The role of IL-6 and possibly troponin I elevation warrants further investigation and if validated in additional studies, they may be of interest as biomarkers of cardiac injury after radiation. This may have clinically meaningful implications and could be part of a risk-stratified approach to patient monitoring after radiation. These biomarkers may identify the patients most at risk for effects to the heart and may also identify those patients most likely to benefit from more aggressive cardiac monitoring in the months and years after the completion of radiation therapy. If they begin to increase even during the course of radiation therapy administration, they might also be incorporated into personalized radiation treatment planning and dose adjustments; these would be worthy avenues of further research.

In addition to seeing no dose effect based on mean heart dose in this study, no significant effect was observed of dose to any of the cardiac substructures on the MRI or blood-based biomarkers we evaluated. Indeed, evidence for substructure importance for cardiac injury remains hypothesized but unconfirmed. There is some evidence that dose to subsegments of the left ventricle and coronary artery have been associated with more frequent cardiac injury, albeit with doses to these substructures that were much higher than seen in our study.[44-46] Accurate and consistent delineation of cardiac substructures remains a challenge and may explain this lack of significance, although cardiac MRI information improves the ability to accurately contour these substructures and was used in this study.[47] Perhaps more likely, the very low doses delivered to the heart seen in this study may indeed be below the threshold required to demonstrate changes on MRI, even using modern sequences and approaches.

A number of limitations need to be considered when interpreting the conclusions of this study. Foremost among them is the limited sample size included. Although significant associations were noted even in this small cohort, several MRI and biomarker parameters showed a trend toward significance that perhaps would be more readily discerned in a larger cohort of patients. This limited patient number constrains the ability to detect smaller yet still clinically relevant changes that may be detected by cardiac MRI or serum-based biomarkers as has been reported by other groups. Referral bias is also a concern as patients had to consent to be part of the study, and thus may not be a true representation of the entire population. Most of the cohort were Caucasian or African American women, with little representation of Asian or Hispanic populations. Also, the very low mean heart doses suggest that in an era of modern radiation treatment planning in which MHD is strictly limited during treatment planning, heart dose from breast radiation can be effectively minimized, and thus may mitigate negative cardiac effects that would have been seen in patients treated more than 5 to 10 years ago. Finally, there was limited follow-up in this study (3 months), and it is unclear whether negative cardiac effects of radiation would become more apparent with longer follow-up, an issue that will need to be addressed in subsequent studies.

Because cardiac toxicity constitutes a meaningful late effect for patients who receive radiation therapy for breast cancer, clinical decision-making at the time of treatment is complex. Tradeoffs must be made with inadequate information, including the extent to which to prioritize the dosimetric coverage of treatment targets to ensure tumor control versus limiting the dose that may be received by the heart. Studies such as the one by Darby et al suggest cardiac toxicity might occur even with low doses of radiation, basing their modeling of the dose-response relationship on comparisons of modeled patients treated with older treatment techniques.[4] The doses were only estimated, however, using a single CT scan of a woman of typical body habitus. Our study is hypothesis generating and contributes complementary data from a more modern data set in which doses overall are much lower, and individual patient doses can definitively be identified. Such findings together may be helpful in identifying patients who may need aggressive cardiac monitoring owing to a higher risk of a cardiac event. The current work seeks to build on existing cardiac dose studies by determining whether imaging or blood biomarkers might further help to identify which patients are at highest risk in the current era in which radiation doses are overall considerably lower than in historical studies. Because the use of MR techniques at 3 time points in this study was not more effective than an analysis of blood-based biomarkers, our findings point to the latter as a more promising possibility of a cost-effective biomarker assessment in this context given the lower cost, broader availability, and greater convenience.

In conclusion, in this left-sided breast cancer cohort, our data showed no significant correlation between radiation dose and either changes on sequential MRI scans or the majority of serum biomarkers studied. A correlation, however, was identified between mean heart dose and 2 promising possible serum biomarkers that merits further investigation: IL-6 and troponin I. Future studies should seek to validate these observations. If these findings are verified in a larger sample, they could have substantial clinical implications, ranging from refinements in radiation planning and delivery in the treatment of early-stage breast cancer to better targeted supportive care and studies of potential early pharmacologic intervention.

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