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Incidence of and Risk Factors for Anthracycline- and Trastuzumab-Associated Cardiotoxicity

A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

by

Esther You Park

2015

ABSTRACT

INCIDENCE OF AND RISK FACTORS FOR ANTHRACYCLINE- AND TRASTUZUMAB-ASSOCIATED CARDIOTOXICITY. Esther Park[1], Laura Skrip*[1], Christos Hatzis*[2], Cary P. Gross[3,4], Maysa Abu-Khalaf[2], Raymond Russell[5]. [1]Yale University, School of Medicine, New Haven, CT; [2]Section of Medical Oncology, Department of Internal Medicine, Yale University, School of Medicine, New Haven, CT; [3]Cancer Outcomes, Public Policy and Effectiveness Research Center; [4]Department of Internal Medicine, Yale University, School of Medicine, New Haven, CT; [5]Section of Cardiology; Department of Internal Medicine, Yale University, School of Medicine, New Haven, CT. *Equal contributors

A major limitation of some of the most effective breast cancer (BC) therapies is the development of heart failure or left ventricular dysfunction. However, knowledge of the risk factors for development of cardiotoxicity from use of current standard BC therapies is incomplete. This study aimed to determine the incidence and predictors of cardiotoxicity associated with anthracycline and trastuzumab treatment.

We retrospectively identified patients in the Yale Nuclear Cardiology database who were diagnosed with BC between 2003 and 2013. The patients were grouped into cohorts based on whether they had received an anthracycline or trastuzumab as a part of their treatment regimen. As some patients had received both of these agents, the cohorts were not mutually exclusive. The independent variables of interest were baseline patient characteristics and comorbidities, baseline cardiac imaging parameters, and treatment factors (radiotherapy, cumulative anthracycline dose, and treatment with other agents). The endpoint was development of cardiotoxicity, as defined by a new diagnosis of congestive heart failure (CHF), admission for an acute CHF exacerbation, or a significant pre-defined decline in left ventricular ejection fraction.

We identified 571 women with BC. In the anthracycline cohort, patients were 50.84 years old on average (SD: 9.65, n = 496). The 3-year cumulative incidences of cardiotoxicity in the anthracycline and trastuzumab cohorts were 12.70 and 42.54 cardiac events per 100 people, respectively. The results of a multivariate Cox proportional-hazards regression analysis suggested that receipt of bevacizumab or trastuzumab independently increased the risk of cardiotoxicity in patients treated with an anthracycline (HR: 4.70, 95% CI for HR: 1.78-12.44, p-value: 0.002 or HR: 10.51, 95% CI for HR: 5.83-18.93, p-value: < 0.001, respectively). In the trastuzumab cohort, the mean age was 52.01 (SD: 10.66, n = 134). In a similar multivariate analysis, anthracycline was not shown to increase the incidence of cardiotoxicity in patients who received trastuzumab, regardless of the cumulative anthracycline dose level (≤ 240 mg/m^2 or > 240 mg/m^2). However, dyslipidemia was a significant risk factor (HR: 3.66, 95% CI for HR: 1.80-7.42, p-value: < 0.001). Interestingly, use of radiotherapy was associated with a lower incidence of developing cardiotoxicity in the anthracycline and trastuzumab cohorts, irrespective of the laterality of radiotherapy. A positive smoking history was related to a shorter time to cardiotoxicity for both the anthracycline and trastuzumab cohorts (HR: 2.82, 95% CI for HR: 1.39-5.71, p-value: 0.0039 and HR: 3.01, 95% CI for HR: 1.42-6.39, p-value: 0.0040, respectively). In the anthracycline cohort, an abnormal baseline left ventricular end-diastolic volume and an abnormal baseline peak filling rate were not significantly associated with cardiotoxicity.

In conclusion, trastuzumab and bevacizumab significantly increase risk of cardiotoxicity in patients who receive an anthracycline. Smoking and dyslipidemia are potential targets for risk reduction. The lack of a significant cardiotoxic effect associated with radiotherapy suggests that previously accepted beliefs regarding radiotherapy's harmful cardiac effects when used for BC treatment may be outdated, although further analysis in larger groups and accounting for additional confounding variables is necessary. Future studies are necessary to re-evaluate modern radiotherapy's cardiac effects.

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INTRODUCTION

Despite improved screening measures and the ability to detect disease at an earlier stage, breast cancer continues to place a significant burden on public health (1). It accounts for 23% of cancer cases and is the most common cause of cancer-related deaths in women globally (2). However, scientific advancements continue to expand our understanding of its pathological characteristics, and we now have a more diverse arsenal of breast cancer treatments. These include, but are not limited to, chemotherapeutic agents such as taxanes, anthracyclines, 5-fluorouracil, capecitabine, and cyclophosphamide; endocrine agents such as aromatase inhibitors and tamoxifen; monoclonal antibodies such as trastuzumab; antiangiogenic compounds such as bevacizumab; and radiotherapy.

Fortunately, these breast cancer treatments have improved disease-free and overall survival (3-6). For instance, the NOAH trial found that in patients with HER2-positive breast cancer who received trastuzumab, the 3-year event-free survival was 71% (3). This was significantly higher than the 3-year event-free survival of 56% in those who had not received trastuzumab (3). A pooled analysis also found that higher cumulative anthracycline doses were significantly associated with pathological complete response (pCR), particularly in HER2-negative patients (7). The same analysis found other factors related to chemotherapy, such as an increased number of chemotherapy cycles in hormone receptor (HR)-positive patients, were significantly associated with pCR as well (7). Though the BEATRICE trial did not demonstrate improved invasive disease-free survival with the addition of bevacizumab to adjuvant therapy in those with triple-negative breast cancer (8), the GeparQuinto trial and NSABP B40 trials did demonstrate significantly increased rates of pCR when bevacizumab was used in the neoadjuvant

setting (9, 10). While data on whether pCR can be used as a reliable surrogate outcome marker at the trial-level have been mixed (11-14), pCR may have an individual-level association with long-term outcome (12, 13). Thus, the positive associations between the mentioned breast cancer treatments and pCR are encouraging.

The benefits of the currently available breast cancer treatment regimens are significant; however, one of the major non-malignant complications of these breast cancer therapies is development of heart failure or ventricular dysfunction (15). While these developments can be asymptomatic, some are life-threatening and dose-limiting (15). Anthracycline is one such chemotherapeutic agent used for multiple types of cancer, including breast cancer (16). Those who receive anthracycline-based therapies for these various cancers are approximately five times as likely to develop cardiotoxicity than those who receive non-anthracycline-based therapies (16). Importantly, asymptomatic cases of cardiotoxicity can progress to irreversible congestive heart failure (CHF) (15). In addition, the survival associated with doxorubicin-induced cardiomyopathy is worse than the survival associated with ischemic cardiomyopathy, one of the most common causes of cardiomyopathy (17).

The putative mechanism behind anthracycline-induced cardiotoxicity continues to be debated. It had long been believed that anthracycline's cardiotoxic effects were mediated by the production of reactive oxygen species (ROS) and increased oxidative stress, ultimately leading to myofibril loss and myocardial vacuolization (18, 19). However, several studies assessing the efficacy of anti-ROS agents such as iron chelators have failed to demonstrate complete protection against doxorubicin-induced cardiotoxicity (19, 20). Rather, the fundamental mechanism of cardiac injury appears to involve the

topoisomerase-II (Top2) enzyme. Top2α is overexpressed in proliferating cells such as cancer cells but otherwise is not normally detectable (18).(21) Doxorubicin binds to this enzyme and DNA, forming a ternary cleavage complex that subsequently activates cell death (18). However, doxorubicin also binds to Top2β, which is normally expressed in adult cardiomyocytes (18, 21, 22). The implications of this interaction have been studied by Zhang et al., who developed a murine model where cardiomyocyte-specific Top2β was deleted (18). They found that this deletion protected cardiomyocytes not only from DNA double-stranded breaks and transcriptome changes promoting ROS formation but also from progressive heart failure following doxorubicin exposure (18).

Trastuzumab is also associated with cardiotoxicity (15). A meta-analysis found that trastuzumab increased the risk of CHF and declines in left ventricular ejection fraction (LVEF) by 5.11 and 1.83 times, respectively, when compared to regimens without trastuzumab (23). The mechanism of trastuzumab-induced cardiotoxicity has not been completely established, but ErbB2 (HER2/neu) signaling has been implicated. In a mouse model, ErbB2-deficient mice developed dilated cardiomyopathy and increased susceptibility to anthracycline-induced cardiotoxicity (24). Other studies in mice also revealed HER2 signaling to have an important role in proper embryonic cardiac development (25, 26).

Risk factors for trastuzumab- and anthracycline-induced cardiotoxicity have been studied, and several have been reported (15, 27, 28). For instance, radiotherapy in patients treated with high-dose anthracycline regimens has been associated with subsequent development of cardiotoxicity (29). Higher cumulative anthracycline doses, lengthier durations of trastuzumab therapy, and concomitant trastuzumab and anthracycline treatment regimens

have also been associated with development of cardiotoxicity (15). Still, the risk factors for developing cardiotoxicity have not been completely defined (30). Previous studies that aimed to identify risk factors may also be outdated, such as those that involved pre-modern radiotherapy techniques (30). Left-sided radiotherapy can expose the heart to a greater mean dose of radiation than right-sided radiotherapy due to the heart's anatomic position (31), and therefore left-sided radiotherapy may increase a patient's risk for developing heart disease. However, this may not be the case in the modern era of radiotherapy, as the incidental radiation to the heart has now been decreased significantly (30). Furthermore, there have been few studies on baseline equilibrium radionuclide angiocardiography (ERNA) imaging parameters as potential predictors of clinically significant anthracycline- or trastuzumab-induced cardiotoxicity. While baseline left ventricular ejection fraction is typically evaluated before a patient begins an anthracycline or trastuzumab, other imaging parameters (such as baseline peak filling rate and left ventricular end diastolic volume) as potential predictors of cardiotoxicity have not been as well studied.

These deficiencies are reflected by the lack of detailed guidelines for noninvasive cardiac monitoring in patients treated with cardiotoxic breast cancer therapies (30). Better characterization of the risk factors and predictors for cardiotoxicity associated with modern-day breast cancer therapies is critical for risk-reduction efforts and for identifying individuals who may need more stringent monitoring of cardiac function. This would allow earlier detection of cardiotoxicity and subsequent intervention in those patients who would most benefit from being subjected to more frequent cardiac evaluations. Early detection of cardiotoxicity is critical since cardioprotective

interventions implemented at the subclinical stage can be more effective than when implemented during its later, symptomatic stages (32).

STATEMENT OF PURPOSE AND HYPOTHESES

In this study, we sought to determine the incidence of and risk factors for cardiotoxicity, as defined by a change in ejection fraction or development of CHF symptoms, associated with current anthracycline- or trastuzumab-based breast cancer therapies. We also aimed to evaluate baseline imaging parameters as potential predictors of cardiotoxicity. The overarching goal was to identify patients who may require risk management and/or more rigorous cardiac monitoring so that they can continue to benefit from the effective anthracycline- or trastuzumab-based breast cancer therapies.

Our hypotheses were the following:

- Given the development of approaches to reduce cardiotoxicity such as more targeted radiotherapy techniques (30), the incidence of anthracycline- and trastuzumab-induced cardiotoxicity is now lower than previously published incidences.
- Increased age, increased BMI, a positive family history of cardiac disease, a positive smoking history, and renal/cardiovascular/liver comorbidities are associated with increased risk for developing anthracycline- or trastuzumab-induced cardiotoxicity.
- Abnormal left ventricular ejection fraction, peak filling rate, and left ventricular end-diastolic volume on baseline equilibrium radionuclide angiocardiography are predictors of cardiotoxicity.

- Left-sided chest radiotherapy is associated with increased risk for developing anthracycline- or trastuzumab-induced cardiotoxicity.
- The risk for developing anthracycline-induced cardiotoxicity increases as the cumulative dose increases.
- Additional treatment (such as trastuzumab, anthracycline, and/or bevacizumab) increases the risk of developing cardiotoxicity in those who receive trastuzumab or anthracycline.

METHODS

Data Sources

Data were obtained from the Yale Nuclear Cardiology database, which contains limited data on patients' characteristics, comorbidities, and equilibrium radionuclide angiocardiography (ERNA) findings. The Yale Nuclear Cardiology database was used for two reasons. Firstly, until approximately two years ago, ERNA was the preferred method of measuring left ventricular systolic and diastolic function in patients receiving chemotherapy at Yale. Secondly, the echocardiography reporting system is not configured to search reliably for patients based on whether they will or have received chemotherapy.

Medical records from Yale-New Haven Hospital, Hospital of Saint Raphael, Yale Smilow Cancer Hospital, and Yale Cancer Care Centers were also reviewed to obtain more complete data about patients' comorbidities, imaging, and treatment histories. The Yale University Human Investigation Committee approved this medical record review (HIC #1303011697). While I performed most of the retrospective chart review, Igor

Medic, MD; Raymond Russell, MD; and Sara Anwar, MD, also participated during the initial stages.

Study Sample

Breast cancer patients who received anthracycline (doxorubicin, Doxil, or epirubicin) and/or trastuzumab as a part of their treatment and whose baseline LVEF was determined by ERNA were included in the study. Patients were excluded if they met any of the following criteria: 1) received any chemotherapy or radiotherapy prior to their baseline ERNA, 2) had a prior diagnosis of a cancer that typically requires chemotherapy or radiotherapy, or 3) received dexrazoxane during chemotherapy for their breast cancer.

Study Design

This was a retrospective study, where the endpoint and outcome variable was cardiotoxicity, as indicated by a cardiac event. Data on baseline patient risk factors and various treatment factors were extracted using a chart abstraction tool that I created for consistency. Patients were sub-grouped into two cohorts. The anthracycline cohort consisted of patients who received an anthracycline, regardless of whether or not trastuzumab was also a part of their treatment regimen. The trastuzumab cohort consisted of patients who received trastuzumab, regardless of whether or not they received an anthracycline. These cohorts were therefore not mutually exclusive. However, they allowed me to identify risk factors specific for developing anthracycline-associated cardiotoxicity and those specific for developing trastuzumab-associated cardiotoxicity in our study sample. The length of follow-up was calculated as the time between the start of chemotherapy, trastuzumab, or bevacizumab and the date of the last clinic/admission note

or imaging study (echocardiogram or ERNA). The time to event was calculated as the time between the start of chemotherapy, trastuzumab, or bevacizumab and the date of a cardiac event.

Study Variables

Baseline patient characteristics and comorbidities

The following baseline patient risk factors were obtained from the Yale Nuclear Cardiology database: age, body mass index (BMI), and family history of cardiac disease. The following baseline patient risk factors were obtained from review of the medical records: history of dyslipidemia, hypertension, diabetes mellitus, smoking, coronary artery disease, valvular heart disease, atrial fibrillation, peripheral vascular disease, liver disease (transient or chronic), stroke or transient ischemic attack, and chronic renal failure. A patient who was a current smoker or had quit smoking in the past year prior to the baseline ERNA study was considered to have a positive smoking history.

Baseline imaging parameters

The following baseline imaging parameters were obtained from ERNA results in the Yale Nuclear Cardiology database: peak filling rate (PFR), left ventricular ejection fraction (LVEF), and LV end-diastolic volume (EDV). Abnormal values for PFR, LVEF, and LV EDV are < 2.5 EDV/sec, < 50%, and ≥140 mL respectively.

Treatment factors

Detailed treatment histories were abstracted from the medical records. They included whether or not the patient received axillary, regional lymph node, and/or chest/breast wall

radiotherapy for her breast cancer. If such radiotherapy was given, the laterality was recorded (right only, left only, or both sides). The cumulative anthracycline dose was also recorded. Epirubicin doses were converted to doxorubicin equivalents according to the Children's Oncology Group Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent, and Young Adult Cancers (Version 3.0) (33).

Additional treatment as a risk factor for cardiotoxicity was also considered. In the anthracycline cohort, additional treatment was considered to be bevacizumab or trastuzumab. None of the patients in our study received both bevacizumab and trastuzumab. The groups in this additional treatment variable were therefore mutually exclusive and consisted of patients who received (1) no additional treatment, (2) bevacizumab, or (3) trastuzumab. In the trastuzumab group, additional treatment was considered to be anthracycline.

Cardiac Event

A cardiac event was defined as or indicated by a new diagnosis of CHF, admission for an acute CHF exacerbation, or a significant decline in LVEF on subsequent evaluations by echocardiography or ERNA. A significant decline in LVEF in a patient with a normal baseline LVEF ($\geq$ 50%) was considered to be a decrease of 10 or more percentage points from baseline or a decrease to below the institution's lower limit of normal (50%). A significant decline in a patient with an abnormal baseline LVEF (< 50%) was considered to be a decrease of 10 or more percentage points from baseline. When a cardiac event could be classified as a decline in LVEF or a new diagnosis of CHF, the former classification was applied.

Assessment of Left Ventricular Function

Parameters of left ventricular function were quantified by ERNA using serial 5- to 7-min acquisitions taken from the left anterior oblique view using a nuclear medicine camera (Myosite, GE Healthcare, Barrington, IL). Red blood cells were labeled *in vitro* with 925-1,110 MBq of ^{99m}Tc-pertechnetate (UltraTag, Mallinckrodt Medical, St. Louis, MO). LV EDV was ascertained using the Massardo count ratio method (34), and LVEF and PFR were calculated using previously validated software based on time activity curves from a region of interest that included the left ventricular cavity (35).

Statistical Analysis

Incidence rates per person-month of follow-up and 3-year cumulative incidences were calculated for each treatment group. Descriptive statistics were generated as frequencies for categorical variables and means and standard deviations for continuous variables. The variables were compared across the treatment groups using the chi-squared test when counts were above five or Fisher's exact test when counts were five or below. All continuous variables were categorized using established cutoff values for normal/abnormal levels in further analyses. Additionally, within each cohort, the number of individuals and number of events were considered for each variable category. Variables with fewer than 5% of the cohort were categorized in any level were excluded from subsequent univariate and multivariate analyses, except for the radiotherapy variable. In this case, rather than removing the entire variable, patients who received bilateral radiotherapy were removed from the multivariate analyses in both cohorts since this group included too few patients to be considered statistically relevant.

Unadjusted Kaplan-Meier curves were generated and between-group results were compared using log rank tests. Cox proportional-hazards models were constructed to evaluate unadjusted relationships between variables meeting the criteria outlined above and the outcome. Those variables shown to have statistically significant associations in the univariate analyses and variables with known clinical significance in relation to occurrence of cardiotoxicity were included in a multivariate Cox proportional-hazards regression model.

To evaluate for the interaction of confounding variables when statistical significance changed between the adjusted and unadjusted analyses, bivariate Cox proportional hazard models were used. Each potential confounder and the variable significantly associated with cardiotoxicity in the multivariate analysis only were modeled together to determine if the adjusted estimate for the effect of the variable was changed relative to the unadjusted estimate. Statistical analyses were performed by Christos Hatzis, PhD, and Laura Skrip, MPH, using R Statistical Software version 3.0.1 (Foundation for Statistical Computing). P-values less than 0.05 were considered statistically significant unless otherwise indicated.

RESULTS

We identified 571 patients in the Yale Nuclear Cardiology database who were diagnosed with breast cancer from 2003 through 2013 and met the criteria for inclusion in the analysis. All patients who qualified for the study were female. Four hundred ninety six patients received an anthracycline, and 134 patients made up the trastuzumab cohort.

Within the anthracycline cohort of 496 patients, 389 (78%) received an anthracycline but no bevacizumab or trastuzumab, 30 (6.0%) received anthracycline + bevacizumab (but no trastuzumab), and 76 (15%) received anthracycline + trastuzumab (but no bevacizumab) (Table 1a). Data on additional treatment were missing for one patient receiving an anthracycline. There were a total of 69 (13.9%) cardiac events in the anthracycline cohort. All of them were due to significant declines in LVEF. The frequency of cardiotoxicity did differ across treatment subgroups in the cohort ($p < 0.001$), with only 6.94% of patients in the anthracycline-only subgroup experiencing an event, but 23.33% in the anthracycline + bevacizumab subgroup and 46.05% in the anthracycline + trastuzumab subgroup experiencing cardiotoxicity.

The overall cardiotoxicity incidence rate and 3-year cumulative incidence in the anthracycline cohort were 0.0032 events/person-month and 12.70 cardiac events per 100 people, respectively (Table 1a). In the anthracycline-only, anthracycline + bevacizumab, and anthracycline + trastuzumab subgroups, the 3-year cumulative incidences were 5.91, 23.33, and 43.42 cardiac events per 100 people, respectively ($p < 0.001$).

Patients in the treatment subgroups of the anthracycline cohort did not significantly differ in terms of proportions of individuals presenting with risk factors, such as history of dyslipidemia, hypertension, diabetes mellitus, smoking, coronary artery disease, valvular heart disease, atrial fibrillation, peripheral vascular disease, liver disease (transient or chronic), stroke or transient ischemia attack, and chronic renal failure (Table 1a). Furthermore, they did not differ in terms of proportions of individuals with family histories of cardiac disease. None of the patients in this cohort had valvular heart disease, and this was not included in Table 1a. The subgroups were likewise similar in terms of

age ($p = 0.75$) and BMI ($p = 0.76$). Most patients in the anthracycline cohort received radiotherapy and were similarly likely to have it on the left side only (34.89%) or right side only (32.66%). This distribution did not significantly vary across treatment subgroups ($p = 0.081$). The mean cumulative anthracycline dose received by patients differed significantly across subgroups ($p = 0.013$), but there was no significance seen when the cumulative anthracycline dose was categorized into ≤ 240 mg/m^2 and > 240 mg/m^2 ($p = 0.11$). The mean cumulative anthracycline dose received was 241.3± 24.75 mg/m^2 (range: 60 mg/m^2-450 mg/m^2).

Table 1b provides a descriptive overview of the trastuzumab cohort. Among the 134 patients receiving trastuzumab, 76 (57%) also received an anthracycline, while 57 (43%) did not receive an anthracycline. Data on additional treatment were missing for one patient receiving trastuzumab. There were a total of 59 (44.03%) cardiac events in the trastuzumab cohort. Two (3%) of these events were indicated by a CHF exacerbation-related hospital admission, while 57 (97%) of them were due to significant declines in LVEF. The proportion of individuals experiencing a cardiac event was over 40% in both groups and did not significantly differ between the two groups ($p = 0.63$). As with the anthracycline cohort, patients receiving trastuzumab did not significantly differ in terms of the frequency of most risk factors considered. It was observed, however, that patients who were on trastuzumab but not an anthracycline were more likely to have a history of chronic renal failure (10.53% versus 1.33%, $p = 0.042$). Furthermore, on average, patients who were on trastuzumab but not an anthracycline were older (55.44 ±11.24 years) than those receiving both trastuzumab and an anthracycline (49.47±9.58 years). Yet, the groups did not significantly differ in terms of the proportions of individuals over

65 years old – the age cutoff associated with increased risk of cardiac events. The mean cumulative anthracycline dose among those who also received an anthracycline was 239.9±19.1 mg/m^2 (range: 120 mg/m^2-310 mg/m^2). None of the patients had valvular heart disease, and this variable was excluded from the Table 1b.

The overall cardiotoxicity incidence rate and 3-year cumulative incidence in the trastuzumab cohort were 0.02 events/person-month and 42.54 cardiac events per 100 people, respectively (Table 1b). In the trastuzumab without anthracycline and trastuzumab with anthracycline subgroups, the 3-year cumulative incidences were 52.27 and 43.42 cardiac events per 100 people, respectively ($p = 0.46$).

Overall, the median time to event was 10.9 months (range: 1.2-91.0), and the median follow-up length in those who did not experience a cardiac event was 48.3 months (range: 0.8-115.6). In the anthracycline cohort, the median time to event was 9.0 months (range: 1.3-91.0), while the median follow-up length in those who did not experience a cardiac event was 47.0 months (range: 0.9-115.6). In the trastuzumab cohort, the median time to event was 9.8 months (range: 1.2-91.0), while the median follow-up length in those who did not experience a cardiac event was 46.4 months (range: 0.8-99.6).

Select Kaplan-Meier curves for significant univariate relationships between the covariate and the outcome variable are shown in Figures 1 and 2. In the anthracycline cohort, there were significant differences in the cardiotoxicity-free survival curves associated with no additional treatment, bevacizumab as additional treatment, and trastuzumab as additional treatment ($p < 0.001$: Figure 1a). Of the three, the group receiving anthracycline and trastuzumab exhibited the worst cardiotoxicity-free survival curve (Figure 1a).

Interestingly, in the trastuzumab cohort, anthracycline treatment was not associated with significantly worse cardiotoxicity-free survival compared to that for patients who did not receive an anthracycline ($p = 0.99$: Figure 2a).

In both the anthracycline and trastuzumab cohorts, left-sided and right-sided radiotherapy were both significantly associated with better cardiotoxicity-free survival compared to no radiotherapy (anthracycline cohort: $p < 0.001$ for left-sided versus none, $p < 0.001$ for right-sided versus none; trastuzumab cohort: $p < 0.001$ for left-sided versus none, $p = 0.023$ for right-sided versus none; Figures 1b and 2b). The laterality of radiotherapy did not affect cardiotoxicity-free survival in either cohort (anthracycline cohort: $p = 0.17$ for left-sided versus right-sided; trastuzumab cohort: $p = 0.15$ for left-sided versus right-sided; Figures 1b and 2b). A history of dyslipidemia at baseline and age > 65 in those who received trastuzumab were associated with increased rates of cardiotoxicity compared to no history of dyslipidemia and age ≤ 65, respectively ($p = 0.010$ and $p < 0.001$, respectively).

In the multivariate Cox proportional-hazards regression analysis of the anthracycline cohort, bevacizumab and trastuzumab significantly increased the risk of cardiotoxicity by 4.70- and 10.51-fold, respectively (HR: 4.70, 95% CI for HR: 1.78-12.44, p-value: 0.0018 and HR: 10.51, 95% CI for HR: 5.83-18.93, p-value: < 0.001, respectively; Table 2). However, both left-sided radiotherapy and right-sided radiotherapy were associated with a significantly lower risk of cardiotoxicity by 0.16 and 0.30 times, respectively (HR: 0.16, 95% CI for HR: 0.07-0.35, p-value: < 0.001 and HR: 0.30, 95% CI for HR: 0.16-0.56, p-value: < 0.001, respectively).

In the multivariate Cox proportional-hazards regression analysis of the trastuzumab cohort, dyslipidemia was significantly associated with developing cardiotoxicity (HR: 3.66, 95% CI for HR: 1.80-7.42, p-value: < 0.001; Table 3). However, dyslipidemia had not been a significant risk factor in the anthracycline cohort (Table 2). Again, left-sided radiotherapy and right-sided radiotherapy were both associated with a significantly lower risk of cardiotoxicity 0.23 and 0.31 times, respectively (HR: 0.23, 95% CI for HR: 0.10-0.52, p-value: < 0.001 and HR: 0.31, 95% CI for HR: 0.15-0.64, p-value: 0.0017, respectively). Although age was a significant risk factor in the unadjusted analysis, it was no longer a significant risk factor after adjusting for the other covariates. Also, as seen in the Kaplan-Meier curves, anthracycline treatment at either of the cumulative dose levels (≤ 240 mg/m^2 or > 240 mg/m^2) did not significantly add to the risk of developing cardiotoxicity.

In both cohorts, smoking history was not a significant risk factor in the univariate analysis (Tables 2 and 3). However, it was a significant risk factor for developing cardiotoxicity after adjustment in the multivariate Cox proportional-hazards regression analysis (Tables 2 and 3). In the anthracycline cohort, the adjusted hazard ratio was 2.82 (95% CI for HR: 1.39-5.71, p-value: 0.0039). In the trastuzumab cohort, the adjusted hazard ratio was 3.01 (95% CI for HR: 1.42-6.39, p-value: 0.0040). In the anthracycline cohort, the smoking effect had been confounded by radiotherapy, which is associated with a lower risk of developing cardiotoxicity in our study. In the trastuzumab cohort, dyslipidemia and age were found to be confounders.

A history of HTN, diabetes mellitus, and liver disease were not significant predictors of developing cardiotoxicity in either cohort (Tables 2 and 3). Body mass index and a family history of cardiac disease were also not significant risk factors.

Baseline imaging factors were evaluated in the anthracycline cohort. For the sample of breast cancer patients receiving an anthracycline, the median LV EF was 63.0% (range: 44.0 – 79.0), median PFR was 3.21 EDV/sec (range: 1.22 – 5.54), and median LV EDV was 101.0 mL (range: 46.0 – 253.0). Abnormal baseline LV EF values were only observed in approximately 1% of the sample, while abnormal PFR and LV EDV levels were found in almost 14% and 16.5% of the sample, respectively. The treatment subgroups did not differ significantly in their relative proportions of individuals with abnormal imaging factor levels (Table 4).

Kaplan-Meier curves for univariate relationships between the baseline imaging factors and the outcome variable in the anthracycline cohort were also generated and compared. An enlarged left ventricular cavity on the baseline study (LV EDV $\geq$140 mL) was associated with a significantly reduced cardiotoxicity-free survival compared to a normal LV EDV less than 140 mL (p-value = 0.030: Figure 3a). However, an abnormal PFR, indicating the presence of abnormal left ventricular relaxation, was not associated with a significantly different cardiotoxicity-free survival curve compared to a normal PFR (p = 0.51: Figure 3b). None of the five individuals with an abnormal LVEF developed cardiotoxicity, but there were not enough individuals with an abnormal LVEF to include in the univariate analyses.

In the univariate Cox proportional-hazards analysis, an abnormal PFR again was not significantly associated with cardiotoxicity in the anthracycline cohort. However, those with an abnormal LV EDV had a significantly faster time to event than those with a normal LV EDV (HR: 1.80, 95% CI for HR: 1.05-3.08, p-value 0.032; Table 5). After adjusting for cumulative anthracycline dose, additional treatment, chest radiotherapy, age, BMI, smoking history, family history of cardiac disease, and comorbidities (hypertension, diabetes, dyslipidemia, and liver disease) in a multivariate Cox proportional-hazards model, neither PFR nor LV EDV was significantly associated with cardiotoxicity (Table 5).

It should be noted that a univariate Cox proportional-hazards analysis was carried out for the trastuzumab cohort as well. However, neither PFR nor LV EDV was significantly associated with the development of cardiotoxicity, and therefore a multivariate analysis was not pursued. Furthermore, there were not enough individuals with an abnormal LVEF from the trastuzumab cohort to include in the Cox proportional-hazards analyses.

DISCUSSION

Several important findings have resulted from the described investigations. Some of the results confirm the conclusions of prior studies, while others challenge previously accepted beliefs. The most unexpected of the latter were those of radiotherapy's association with decreased rates of cardiotoxicity.

Radiotherapy for breast cancer has been known to be potentially cardiotoxic, as supported by large meta-analyses of randomized trials (36, 37). Both Cuzick et al. and the Early Breast Cancer Trialists' Collaborative Group found that radiotherapy increased

cardiac or vascular mortality, respectively, when compared to breast cancer patients who didn't receive radiotherapy (36, 37). However, most of the trials included in these meta-analyses utilized pre-modern radiotherapy techniques, which delivered greater amounts of radiation to the heart (30, 36, 37). Since around 1985, more modern techniques such as image-guided therapy, reduction of field size, and respiratory gating have been used to reduce incidental cardiac irradiation (30).

Tumor registry studies have demonstrated the impact of these changes in radiotherapy techniques over time (31, 38). Giordano et al. examined three time periods during which patients were diagnosed with breast cancer: 1973-1979, 1980-1984, and 1985-1989 (31). During the first time period, they found that those who received left-sided radiotherapy had a 15-year mortality from ischemic heart disease of 13.1%, which was significantly different from the 10.2% 15-year mortality of those who received right-sided radiotherapy (31). However, in the two latter time periods, no mortality differences were observed based on laterality of radiotherapy (31). Furthermore, 15-year mortality decreased from 13.1% to 5.8% over the three time periods in those who received left-sided radiotherapy. These findings were presumably due at least in part to changing radiotherapy techniques that delivered less radiation to the heart (31). Left-sided radiotherapy exposes the heart to a greater mean dose of radiation than right-sided radiotherapy due to the heart's anatomic position (31). However, Giordano's findings suggest that with changing radiotherapy techniques over time, radiation exposure from left-sided radiotherapy has decreased such that it no longer confers a significantly higher risk of mortality from ischemic heart disease than right-sided radiotherapy. Darby et al. had similar findings, with patients who received left-sided radiation between 1973 and

1982 experiencing significantly higher cardiac mortality rates than those who received right-sided radiation (38). These differences were no longer significant in later time periods (38).

Findings from other recent studies on breast cancer patients have been conflicting. Hojris et al. performed an analysis of two randomized trials and found that radiotherapy did not increase the risk of ischemic heart disease, including myocardial infarction (39). Doyle et al. focused their study on women ≥65 years of age and found that the risk of myocardial infarction likewise did not increase with receipt of radiotherapy, regardless of tumor laterality (40). On the other hand, McGale et al. demonstrated in a prospective study that left-sided radiotherapy increases the risk for heart disease, relative to right-sided radiotherapy, even for women diagnosed with breast cancer after 1990, a time during which they would have presumably been treated with more modern radiotherapy techniques (41). According to Darby et al., even low doses of radiotherapy are associated with increased risk for ischemic heart disease (42).

It should be mentioned, though, that these studies are not comparable to ours. Unlike our study, the outcome of interest in many of these analyses was cardiac ischemia, which is presumably due to the mechanism of radiotherapy-induced cardiotoxicity. Radiotherapy is known to induce atherosclerosis, particularly at higher doses (43). Still, radiotherapy can also damage the microvasculature and promote inflammatory changes and interstitial fibrosis, potentially resulting in congestive heart failure (43).

There have been studies that explored the effects of radiotherapy on subsequent development of heart failure or decreases in LVEF in breast cancer patients, though these

are not as frequent as those on cardiac ischemia (44-48). Some have found that radiotherapy does not increase the risk of developing these cardiac outcomes (44, 46, 48), while others have found that they do increase the risk (45, 47). However, these studies differed from ours in that not all of the patients received chemotherapy (44), patients were treated with radiotherapy in an earlier time period (1970-1986) (45), or the patients received trastuzumab and radiotherapy concurrently (46-48). At our institution, trastuzumab is typically held during courses of radiotherapy.

Our study is unique in that modern radiotherapy, regardless of laterality, was associated with significant improved cardiac outcomes in breast cancer patients who received an anthracycline and/or trastuzumab. Reports of radiotherapy having a cardioprotective effect in breast cancer patients are rare. We have only encountered one such report (49), and even then, it was not presented as one of the main results. Specifically, Du et al. found that patients $\geq$ 65 years of age who receive radiotherapy are less likely to develop congestive heart failure than those who do not (49).

Despite the lack of pre-existing evidence, the idea that radiotherapy can be cardioprotective is not entirely far-fetched. Experiments have demonstrated that low doses of <1 Gy can have anti-inflammatory effects (50, 51). In hypercholesterolemic mice, exposure to low doses of radiation is associated with reductions in the numbers and sizes of atherosclerotic lesions and in total serum cholesterol levels (52). It is possible that the cardioprotective effect seen in our study was possibly due to a preconditioning effect, akin to ischemic preconditioning where brief periods of ischemia can protect against more prolonged insults (53). Low doses of radiation to the heart may have protected it against further insults from chemotherapy and/or trastuzumab.

Still, the cardioprotective effects seen in our study should be considered with caution, as there may be potential confounding with factors such as additional treatment type. In the anthracycline cohort (excluding those who got bilateral radiotherapy since they had also been excluded from the multivariate analyses), 20 (27%) of the 74 patients treated with trastuzumab received left-sided radiotherapy. However, 152 (38%) of the 400 patients who weren't treated with trastuzumab received left-sided radiotherapy. This bias almost reaches significance (Fisher's exact test p-value = 0.09). Given that trastuzumab (as will be further discussed later) exhibited a very significant cardiotoxic effect in our study, this potential confounding finding could thus have produced the cardioprotective effect.

However, this reasoning fails when applied to right-sided radiotherapy. In the same anthracycline cohort (again excluding those who received bilateral radiotherapy), 24 (32%) of the 74 patients treated with trastuzumab underwent right-sided radiotherapy. Similarly, 136 (34%) of the 400 patients who were not treated with trastuzumab were given right-sided radiotherapy (Fisher's exact test p-value = 0.89). There is no clear bias in this case that could explain the significant cardioprotective effect exhibited by right-sided radiotherapy.

Furthermore, the trastuzumab cohort does not lend itself to a similar evaluation of potential confounding with additional treatment type since all of the patients had received trastuzumab. As will be discussed later, additional anthracycline treatment is not associated with increased risk of cardiotoxicity in this cohort. Therefore, any differences in the proportions of patients given radiotherapy who did or did not receive anthracycline treatment in addition to trastuzumab is not of relevance in this particular discussion.

Though the observed beneficial effects cannot be completely attributed to confounding by additional treatment, the bias seen in the anthracycline cohort is a reminder of the limitations of a retrospective study. There may be several other confounding factors that we were unable to adjust for in this study. As such, it would be premature to definitely conclude that radiotherapy is cardioprotective based on our findings. Rather, until our findings can be reproduced by other studies and trials, emphasis should be placed instead on the lack of a significant cardiotoxic effect associated with radiotherapy. The absence of this relationship in our results suggests that previously accepted beliefs regarding radiotherapy's harmful cardiac effects may be outdated. There is a need for newer studies determining the effects of modern radiotherapy on the heart in the setting of contemporary breast cancer treatments.

As discussed above, another finding of our study is that certain treatment agents add to cardiotoxicity risk. It has been well established that trastuzumab increases the risk of developing cardiotoxicity when given to patients who also receive anthracycline, as we confirmed in our study (48, 49, 54, 55). However, anthracycline at either cumulative dose levels ($\leq$240 mg/m^2 or >240 mg/m^2) did not add to the risk of developing cardiotoxicity in the trastuzumab cohort, when compared to the no-anthracycline subgroup. This is contrary to previous findings of increased risk with treatments based on both anthracycline and trastuzumab (56), and we suspect that the negative finding was perhaps partly due to a treatment bias. Patients who were not given both trastuzumab and anthracycline may have had a greater predisposition for developing cardiotoxicity than those who did receive both. There may have been aspects of their medical history that

discouraged clinicians from treating patients who had to receive trastuzumab in addition to an anthracycline.

Similarly, the 3-year cumulative incidence of cardiac events was higher in the anthracycline plus trastuzumab subgroup of the anthracycline cohort than in the anthracycline-only subgroup (43.42 cardiac events per 100 people versus 5.91 cardiac events per 100 people). However, in the trastuzumab cohort, there was no significant difference between the trastuzumab without anthracycline (52.27 cardiac events per 100 people) and trastuzumab plus anthracycline subgroups (43.42 cardiac events per 100 people) ($p = 0.46$).

The incidences observed in our study are generally higher than those published in other studies. For example, the observed 3-year cumulative incidences of heart failure or cardiomyopathy in a retrospective study of elderly women were 28.2, 15.3, and 26.7 per 100 patients in its anthracycline plus trastuzumab, anthracycline (without trastuzumab), and trastuzumab (without anthracycline) groups, respectively (54). The Herceptin Adjuvant (HERA) trial found the incidence of left ventricular dysfunction at 3.6 years of follow-up to be just 9.8% in those who received trastuzumab (57). The North Central Center Treatment Group (NCCTG) N9831 trial observed even lower cumulative incidences of 0.3% in the anthracycline (without trastuzumab) group and 2.8% in the sequential anthracycline plus trastuzumab (plus paclitaxel) group at 3 years (58). Similarly, the 5-year cumulative incidence in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 trial was 0.9% in the anthracycline (without trastuzumab) group and 3.8% in the sequential anthracycline plus trastuzumab/paclitaxel group (58). The Breast Cancer International Research Group 006 trial likewise observed lower

incidences of cardiotoxicity despite having a longer mean length of follow-up (56). However, unlike our study, these studies did not include subclinical cardiotoxicity in their definition of a cardiac endpoint (54) or excluded those with cardiovascular disease (57-59). We suspect that our findings are closer to the true incidence of cardiotoxicity in anthracycline and/or trastuzumab-treated breast cancer patients.

Bevacizumab was another therapeutic agent that significantly increased the risk of developing cardiotoxicity in the anthracycline cohort. The data regarding this agent's cardiac effects are mixed, with some studies finding that it increases the risk for congestive heart failure (60, 61), while others reporting that it does not (62). It will be important to study further bevacizumab's cardiac toxicities as it has been an agent of interest due to findings that it increases the pathologic complete response rate and improves progression-free survival (63, 64).

Additionally, we found that dyslipidemia was a significant risk factor of cardiotoxicity in the trastuzumab cohort. A positive smoking history was a significant predictor in both the trastuzumab and anthracycline cohorts. Previous studies, including the NSABP B-31 and HERA trials, did not find them to be significant risk factors for the development of CHF (48, 65, 66). However, smoking was a positive predictor for trastuzumab mediated cardiotoxicity in a retrospective study (67). A speckle tracking echocardiographic study also found that smoking negatively affected longitudinal strain in breast cancer patients who received chemotherapy (68). It is suspected that smoking may contribute to cardiotoxicity by increasing oxidative stress (68). In the majority of these previous studies, it is unclear what was considered to be a positive smoking history. In our study, current smokers and those who had quit within the year prior to their baseline ERNA

were considered to have a positive smoking history. Patients who had quit smoking more than a year prior to their baseline ERNA were considered to have a negative smoking history. As such, our findings suggest that recent or current smoking is a potential target for risk reduction and lifestyle modification, as well as dyslipidemia.

A cumulative anthracycline dose > 240 mg/m^2 (240 mg/m^2 is the cumulative dose typically used to treat breast cancer) was a negative predictor for cardiotoxicity. However, it has been well established that higher doses, particularly those greater than 550 mg/m^2, are associated with increased risk (15). Our finding is likely due to the study not being sufficiently powered to detect a difference between the two levels. Only a small proportion of patients had received cumulative anthracycline doses > 240 mg/m^2 (7.28% in the anthracycline cohort and 7.04% of those who received anthracycline in the trastuzumab cohort: Tables 1a and 1b). Furthermore, the highest cumulative anthracycline dose in the anthracycline cohort was just 450 mg/m^2, while the highest cumulative anthracycline dose in the trastuzumab cohort was 310 mg/m^2.

An abnormal baseline LV EDV was significantly associated with cardiotoxicity in the univariate Cox proportional-hazards analysis of the anthracycline cohort. However, after adjustment for various patient and treatment-related factors, the association was no longer significant. An abnormal baseline PFR, a marker of diastolic dysfunction, was not significantly associated with cardiotoxicity in either the univariate or multivariate analysis. Unfortunately, there are no other studies on abnormal baseline ERNA imaging factors that predict cardiotoxicity. However, there have been studies that have assessed various imaging parameters obtained during cancer treatment or post-treatment as subclinical predictors of subsequent significant changes in LVEF or heart failure (in

contrast to assessing pre-treatment ERNA imaging parameters as predictors of developing cardiotoxicity such as in our study). Though the questions addressed by these studies and ours are fundamentally different, we can use the findings of the former as a starting point for discussion.

There have been studies that have focused on early diastolic abnormalities as potential predictors of cardiotoxicity. A case series of three patients who received doxorubicin found that a decrease in the PFR preceded development of changes in LVEF and the development of CHF (69). The authors therefore proposed PFR as a more sensitive marker of early cardiotoxicity than the conventional marker, LVEF (69). Hashimoto et al. also found that the PFR was significantly lower in pediatric patients who received anthracycline compared to a control group, even though there was no significant difference in LVEF between the two groups (70). However, they did not determine whether this potential marker of subclinical cardiotoxicity could be used as a predictor of subsequent changes in LVEF or CHF (70).

The aforementioned studies suggest that chemotherapy-induced cardiotoxicity may be a step-wise process whereby the myocardium undergoes several phases of injury, with diastolic changes perhaps preceding systolic changes. If so, it would seem sensible that patients with baseline abnormalities in PFR or LV EDV prior to beginning anthracycline- or trastuzumab-based treatment would be predisposed to developing cardiotoxicity (as defined by significant declines LVEF or CHF manifestations) due to pre-existing diastolic abnormalities. However, this hypothesis did not hold up in our study. This may be at least partly explained by some important differences between previous studies and ours. Namely, the cancer types being treated in previous studies were varied and

included diagnoses such as rhabdomyosarcoma, acute lymphoblastic leukemia, Wilm's tumor, breast carcinoma, and small cell lung carcinoma (69, 70). Therefore, the cumulative doses of anthracycline that patients received in the case series (69) (mean: 385 ± 105 mg/m^2) and the study by Hashimoto et al. (70) (mean: 305 ± 236 mg/m^2) were on average higher than the cumulative anthracycline doses that our patients received (mean: 241.3 ± 24.75 mg/m^2). It may be that the typical cumulative anthracycline doses administered during the treatment of breast cancer, such as in our study sample, are not high enough to cause significant enough additional diastolic damage. Regardless, our findings suggest that a PFR < 2.5 EDV/sec and an LV EDV ≥140 mL are not strong predictors of cardiotoxicity in breast cancer patients who receive anthracycline- and/or trastuzumab-based treatments.

It would be reasonable to expect an abnormal baseline LVEF to be a predictor of cardiotoxicity, particularly since the definition of cardiotoxicity in our study includes significant declines in LVEF. However, a limitation of our study was that there were only a few patients with an abnormal baseline LVEF, and meaningful statistical analyses of their potential implications could not be carried out. Based on a recent systematic review, an early 10%-15% reduction in peak systolic global longitudinal strain (GLS), as determined by speckle tracking echocardiography (STE), seems to be a valuable predictor of subsequent LVEF declines or heart failure in patients who received trastuzumab and/or anthracycline (32). Such findings highlight the importance of studying baseline LVEF as a potential predictor of cardiotoxicity in future studies that are sufficiently powered to do so.

Future studies should also focus on further examination of the effects of radiotherapy, bevacizumab, dyslipidemia, and smoking on risk of developing cardiotoxicity in breast cancer patients. They are potentially important for risk stratification and reduction. In particular, our findings on radiotherapy illustrate that its effects are not simply cardiotoxic, but that it has a complicated relationship with other treatment factors. It seems that modern radiotherapy techniques that deliver low doses of radiation to the heart, when given after relatively low cumulative doses of anthracycline typical of standard breast cancer chemotherapy, can be potentially cardioprotective against left ventricular systolic dysfunction and heart failure. However, we can only be speculative at this point given the potential for confounding factors and instead place more confidence on the lack of a significant cardiotoxic effect associated with radiotherapy. More studies are necessary to assess the validity of our results and further explore the relationship between low doses of radiotherapy and development of congestive heart failure or systolic dysfunction in breast cancer patients. Future investigations should stratify radiotherapy not just by laterality but also by region and total dose of radiation to the heart. It will also be important to determine whether or not the potential cardioprotective effects of radiotherapy seen in our study, if reproducible, are long-term and persist beyond the follow-up duration of our study. In addition, it will be important to see if there is a linear relationship between cigarette pack-years and risk of developing cardiotoxicity and if there is a minimum pack-year level beyond which risk begins to increase. Elucidating the effects of statin therapy and disease control on dyslipidemia's contributions to cardiotoxicity will also be important.

Study limitations

The greatest limitation of our study was that it was retrospective. However, we attempted to minimize confounding factors and bias by adjusting for many potential confounders and appropriately limiting our sample. Patients who had previously received chemotherapy and/or radiotherapy or were diagnosed with a cancer that would have required either were excluded. Secondly, the study relied on the identification of a cohort of patients based on determination of the baseline LVEF by ERNA. Therefore, patients whose baseline LVEF was determined by echocardiogram were not included. However, based on referral patterns at our institution, we have no reason to believe that there would be significant baseline differences between those who underwent an ERNA versus an echocardiogram. As mentioned previously, the small sample size of patients with an abnormal baseline LVEF was another limitation that prevented meaningful statistical analysis of its association with developing cardiotoxicity.

Conclusions

In both the anthracycline and trastuzumab cohorts of breast cancer patients, radiotherapy was associated with a lower risk of a subsequent decrease in LVEF or development of CHF. However, there may have been confounding factors that this study could not adjust for, and emphasis should instead be placed on the lack of a significant cardiotoxic effect associated with radiotherapy. Dyslipidemia and a positive smoking history are significant predictors for the development of cardiac events and potential targets for risk reduction. Trastuzumab and bevacizumab independently increased the risk of cardiotoxicity in patients who received an anthracycline. Abnormal baseline LV EDV and PFR do not appear to be strong predictors of anthracycline- or trastuzumab-induced cardiotoxicity. Patients with the aforementioned significant risk factors may benefit from

more stringent cardiac monitoring for earlier detection of subclinical myocardial injury so that the benefits of receiving effective breast cancer treatments such as anthracyclines or trastuzumab will outweigh the costs of cardiotoxicity. Future studies are necessary to establish such benefits and determine the optimal monitoring parameters and frequency.

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FIGURES

Figure 1a. Kaplan-Meier Analysis of the Incidence of Cardiotoxicity in the Anthracycline Cohort Based on Additional Treatment

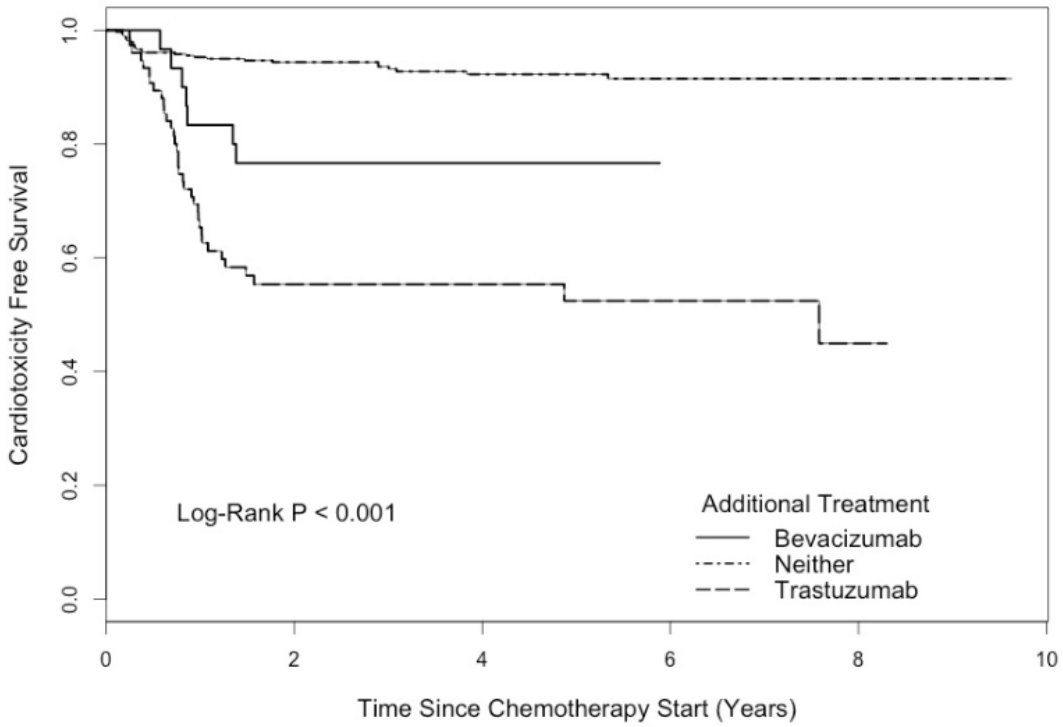


Figure 1b. Kaplan-Meier Analysis of the Incidence of Cardiotoxicity in the Anthracycline Cohort Based on Radiotherapy Location

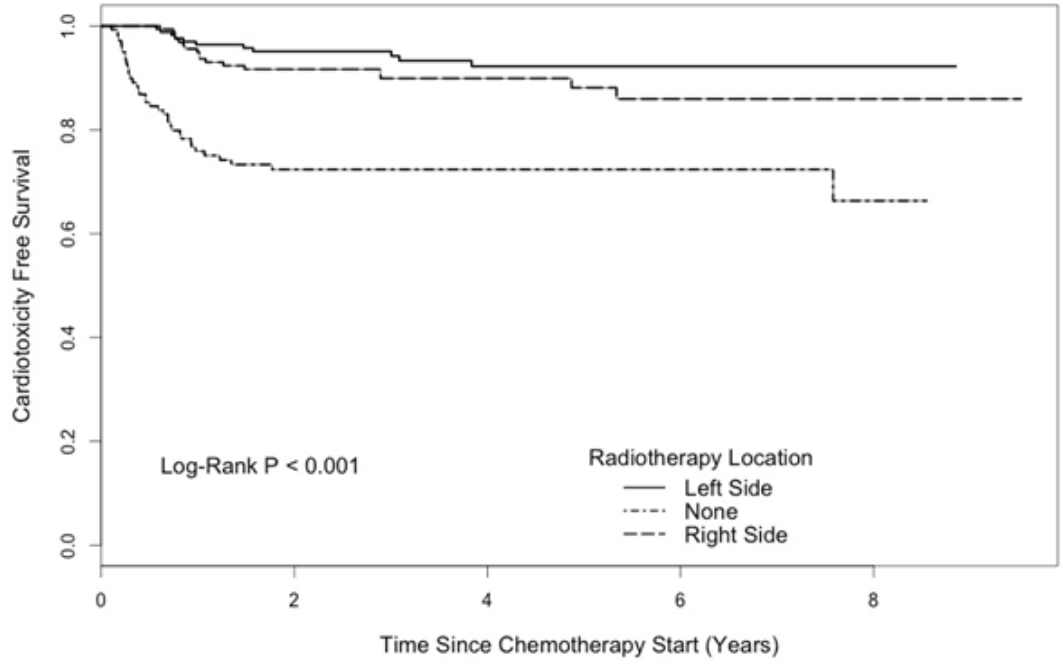


Figure 2a. Kaplan-Meier Analysis of the Incidence of Cardiotoxicity in the Trastuzumab Cohort Based on Additional Treatment

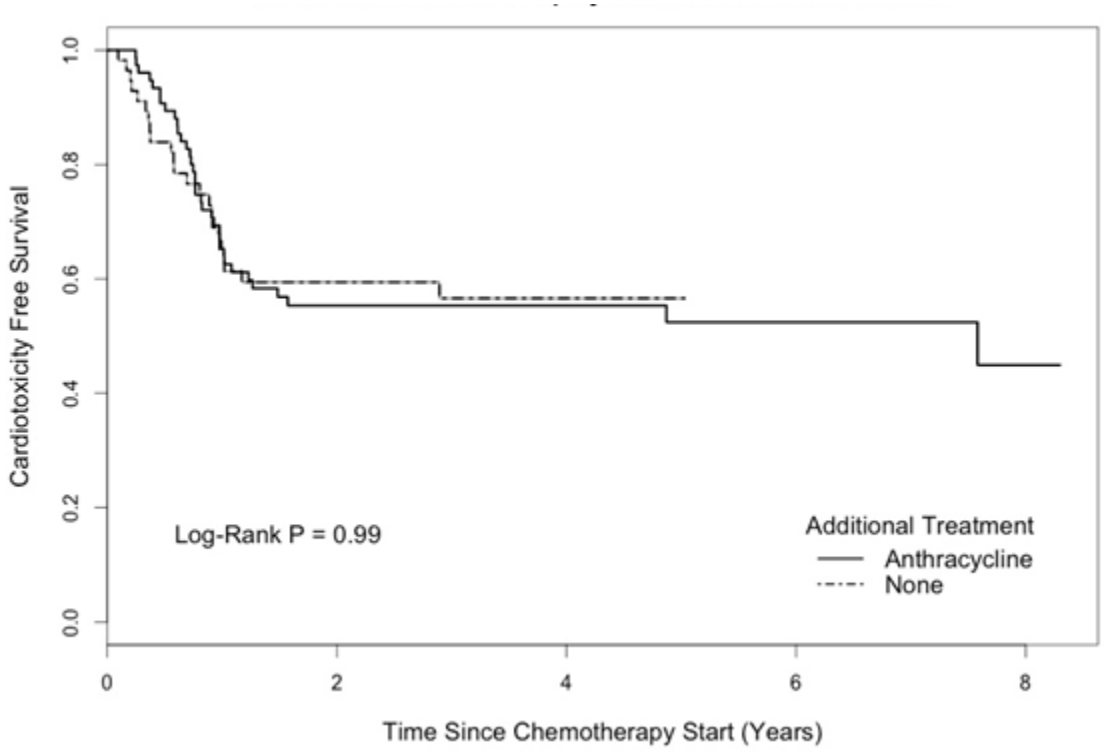


Figure 2b. Kaplan-Meier Analysis of the Incidence of Cardiotoxicity in the Trastuzumab Cohort Based on Radiotherapy Location

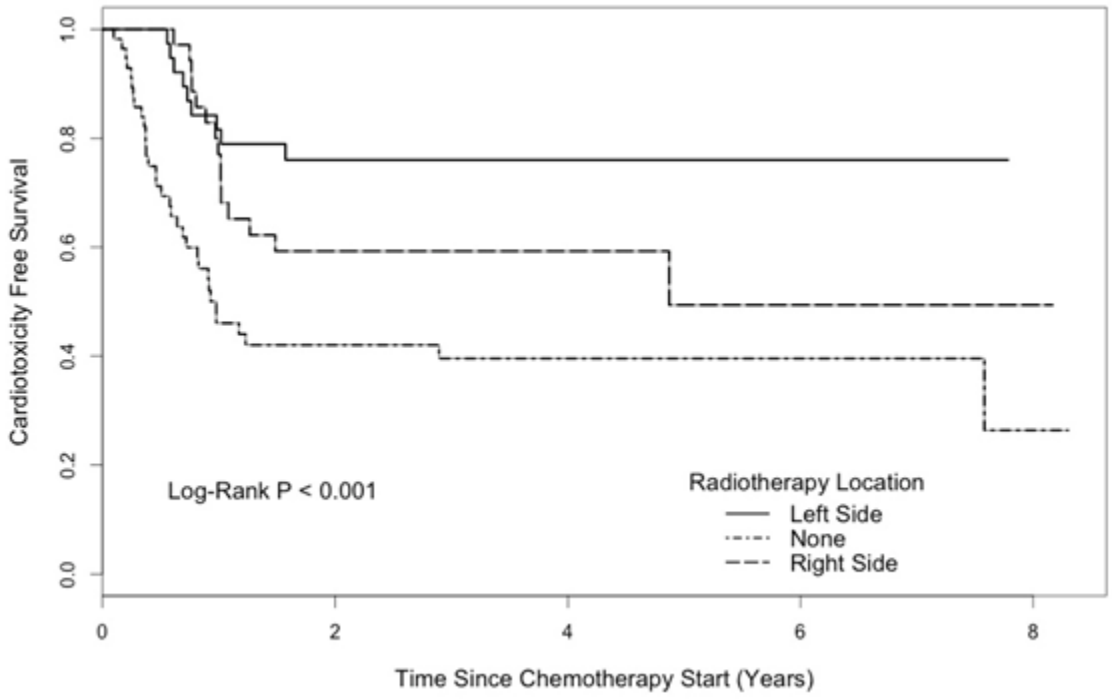


Figure 3a. Kaplan-Meier Analysis of the Incidence of Cardiotoxicity in the Anthracycline Group Based on Left Ventricular End-Diastolic Volume (LV EDV)

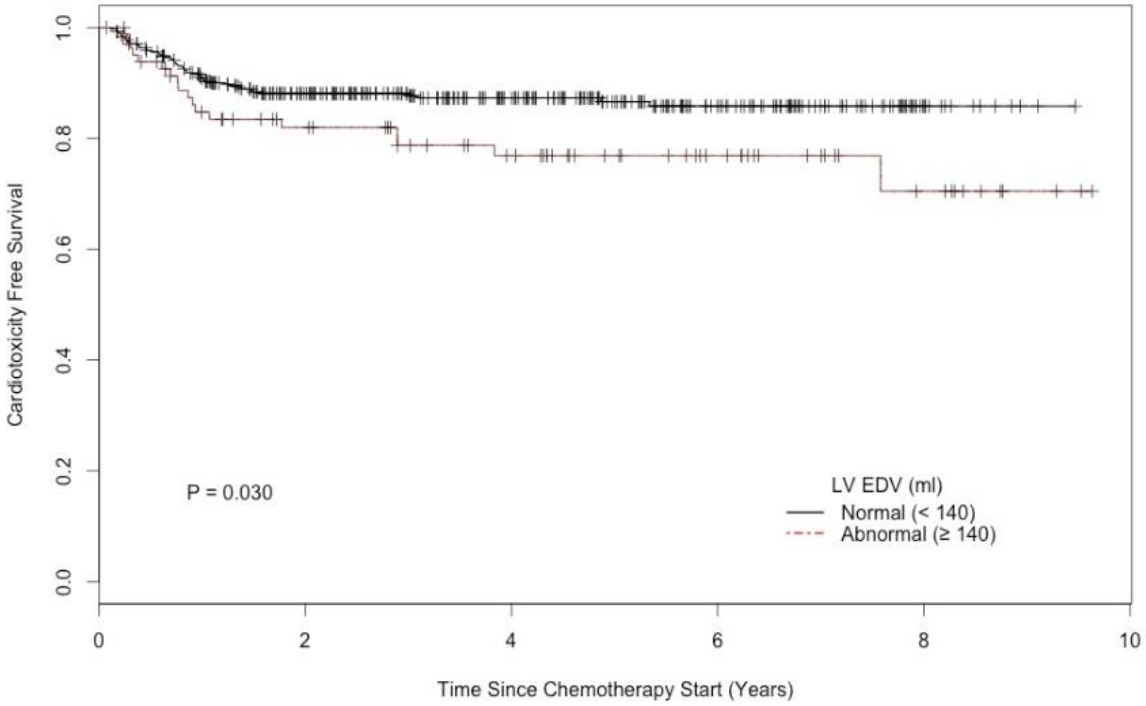
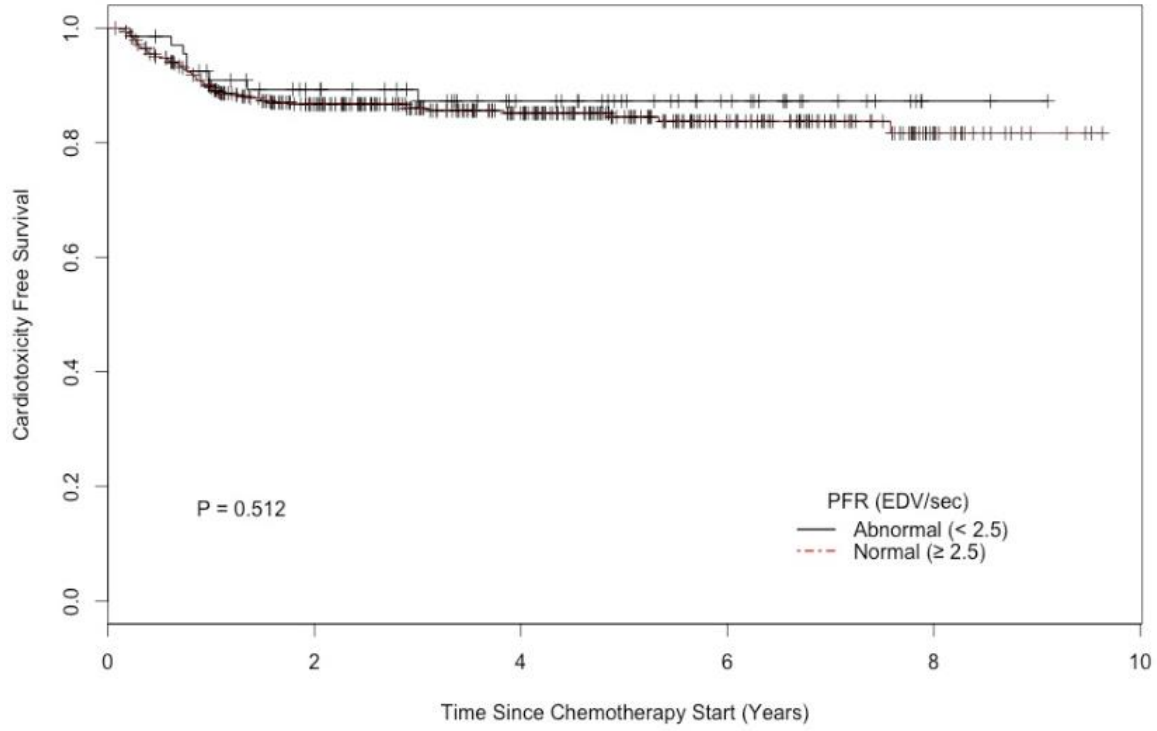


Figure 3b. Kaplan-Meier Analysis of the Incidence of Cardiotoxicity in the Anthracycline Group Based on Peak Filling Rate (PFR)



TABLES

Table 1a. Descriptive Summary of the Anthracycline Cohort

	All Breast Cancer Patients Receiving Anthracycline	Anthracycline - Bevacizumab - Trastuzumab	Anthracycline + Bevacizumab	Anthracycline + Trastuzumab	p-value
Characteristic	**n = 496**	**n = 389**	**n = 30**	**n = 76**	
Incidence Rate (events/person-month)	0.0032	0.0015	0.0068	0.014	<0.001*
3-Year Cumulative Incidence (per 100 people)	12.70	5.91	23.33	43.42	<0.001*
Cardiotoxic Event					
Event	69 (13.9)	27 (6.94)	7 (23.33)	35 (46.05)	<0.001*
No Event	427 (86.1)	362 (93.06)	23 (76.67)	41 (53.95)	
Cumulative Anthracycline Dose (mg/m^2)					
Mean ± SD	241.3 ± 24.75	240.5 ± 23.08	254.4 ± 45.76	239.9 ± 19.11	0.013*
≤ 240	420 (92.72)	330 (93.48)	24 (82.76)	66 (92.96)	0.11
> 240	33 (7.28)	23 (6.52)	5 (17.24)	5 (7.04)	
Radiotherapy					
Left-sided only	172 (34.89)	140 (36.18)	12 (40.00)	20 (26.67)	0.081
Right-sided only	161 (32.66)	126 (32.56)	10 (33.33)	24 (32.00)	
Both left- and right-sided	18 (3.65)	14 (3.62)	3 (10.00)	1 (1.33)	
None	142 (28.80)	107 (27.65)	5 (16.67)	30 (40.00)	
Age (years)					
Mean ± SD	50.84 ± 9.65	51.27 ± 9.59	48.53 ± 10.29	49.47 ± 9.58	0.13
≤ 65	462 (93.15)	360 (92.54)	29 (96.67)	72 (94.74)	0.75
> 65	34 (6.85)	29 (7.46)	1 (3.33)	4 (5.26)	

Table 1a continued

BMI					
Mean ± SD	27.97 ± 6.88	28.14 ± 7.00	27.54 ± 5.47	27.06 ± 6.67	0.45
Normal	183 (37.97)	144 (37.70)	9 (31.03)	30 (42.86)	0.76
Overweight	156 (32.37)	123 (32.20)	12 (41.38)	21 (30.00)	
Obese	143 (29.67)	115 (30.10)	8 (27.59)	19 (27.14)	
Hypertension					
Yes	149 (30.41)	123 (31.95)	9 (30.00)	17 (22.67)	0.28
No	341 (69.59)	262 (68.05)	21 (70.00)	58 (77.33)	
Diabetes Mellitus					
Yes	44 (8.96)	36 (9.33)	1 (3.33)	7 (9.33)	0.69
No	447 (91.04)	350 (90.67)	29 (96.67)	68 (90.67)	
Dyslipidemia					
Yes	114 (23.27)	91 (23.64)	5 (16.67)	18 (24.00)	0.68
No	376 (76.73)	294 (76.36)	25 (83.33)	57 (76.00)	
History of Smoking					
Yes	80 (16.33)	61 (15.89)	6 (20.00)	13 (17.33)	0.74
No	410 (83.67)	323 (84.11)	24 (80.00)	62 (82.67)	
Family History of Cardiac Disease					
Yes	230 (46.75)	185 (47.80)	12 (40.00)	32 (43.24)	0.58
No	262 (53.25)	202 (52.20)	18 (60.00)	42 (56.76)	
Coronary Artery Disease					
Yes	5 (1.02)	4 (1.04)	0 (0.00)	1 (1.33)	0.70
No	485 (98.98)	381 (98.96)	30 (100.00)	74 (98.67)	

Table 1a continued

Atrial fibrillation					
Yes	1 (0.20)	1 (0.26)	0 (0.00)	0 (0.00)	1.0
No	489 (99.80)	384 (99.74)	30 (100.00)	75 (100.00)	
No	489 (99.80)	385 (100.00)	30 (100.00)	74 (98.67)	
Liver Disease					
Chronic	2 (0.41)	2 (0.52)	0 (0.00)	0 (0.00)	0.95
Transient	43 (8.78)	34 (8.83)	3 (10.00)	6 (8.00)	
None	445 (90.82)	349 (90.65)	27 (90.00)	69 (92.00)	
Stroke/TIA					
Yes	6 (1.22)	4 (1.04)	0 (0.00)	2 (2.67)	0.49
No	485 (98.78)	382 (98.96)	30 (100.00)	73 (97.33)	
Chronic renal failure					
Yes	5 (1.02)	4 (1.04)	0 (0.00)	1 (1.33)	0.70
No	485 (98.98)	381 (98.96)	30 (100.00)	74 (98.67)	

Note: Data are n (%) of persons for whom data are available. Percentages may not sum to one hundred due to rounding.

* Statistically significant at the $p < 0.05$ level

BMI = body mass index; TIA = transient ischemic attack; SD = standard deviation

Table 1b. Descriptive Summary of the Trastuzumab Cohort

	All Breast Cancer Patients Receiving Trastuzumab	Trastuzumab without Anthracycline	Trastuzumab with Anthracycline	p-value
Characteristic	**n = 134**	**n = 57**	**n = 76**	
Incidence Rate (events/person-month)	0.015	0.017	0.014	0.46
3-Year Cumulative Incidence (per 100 people)	42.54	52.27	43.42	0.46
Cardiotoxic Event				
Event	59 (44.03)	23 (40.35)	35 (46.05)	0.63
No Event	75 (55.97)	34 (59.65)	41 (53.95)	
Radiotherapy				
Left-sided only	38 (28.79)	18 (31.58)	20 (26.67)	0.42
Right-sided only	35 (26.52)	11 (19.30)	24 (32.00)	
Both left- and right-sided	2 (1.52)	1 (1.75)	1 (1.33)	
None	57 (43.18)	27 (47.37)	30 (40.00)	
Age (years)				
Mean ± SD	52.01 ± 10.66	55.44 ± 11.24	49.47 ± 9.58	0.0017*
≤ 65	122 (91.04)	49 (85.96)	72 (94.74)	0.12
> 65	12 (8.96)	8 (14.04)	4 (5.26)	
BMI				
Mean ± SD	26.92 ± 6.28	26.69 ± 5.86	27.06 ± 6.67	0.74
Normal	55 (42.97)	25 (43.86)	30 (42.86)	0.75
Overweight	42 (32.81)	21 (36.84)	21 (30.00)	
Obese	31 (24.22)	11 (19.30)	9 (27.14)	

Table 1b continued

Hypertension				
Yes	38 (28.57)	21 (36.84)	17 (22.67)	0.11
No	95 (71.43)	36 (63.16)	58 (77.33)	
Diabetes Mellitus				
Yes	10 (7.52)	3 (5.26)	7 (9.33)	0.51
No	123 (92.48)	54 (94.74)	68 (90.67)	
Dyslipidemia				
Yes	33 (24.81)	15 (26.32)	18 (24.00)	0.92
No	100 (75.19)	42 (73.68)	57 (76.00)	
History of Smoking				
Yes	24 (18.05)	11 (19.30)	13 (17.33)	0.95
No	109 (81.95)	46 (80.70)	62 (82.67)	
Family History of Cardiac Disease				
Yes	61 (47.67)	29 (54.72)	32 (43.24)	0.27
No	67 (52.34)	24 (45.28)	42 (56.76)	
Coronary Artery Disease				
Yes	2 (1.50)	1 (1.75)	1 (1.33)	1.0
No	131 (98.50)	56 (98.25)	74 (98.67)	
Atrial fibrillation				
Yes	1 (0.75)	1 (1.75)	0 (0.00)	0.43
No	132 (99.25)	56 (98.25)	75 (100.00)	
Peripheral vascular disease				
Yes	1 (0.75)	0 (0.00)	1 (1.33)	1.0
No	132 (99.25)	57 (100.00)	74 (98.67)	

Table 1b continued

Liver Disease				
Chronic	1 (0.75)	1 (1.75)	0 (0.00)	0.28
Transient	8 (6.02)	2 (3.51)	6 (8.00)	
None	124 (93.23)	54 (94.74)	69 (92.00)	
Stroke/TIA				
Yes	3 (2.26)	1 (1.75)	2 (2.67)	1.0
No	130 (97.74)	56 (98.25)	73 (97.33)	
Chronic renal failure				
Yes	7 (5.26)	6 (10.53)	1 (1.33)	0.042*
No	126 (94.74)	51 (89.47)	74 (98.67)	

Note: Data are n (%) of persons for whom data are available. Percentages may not sum to one hundred due to rounding.

* Statistically significant at the $p < 0.05$ level

BMI = body mass index; TIA = transient ischemic attack; SD = standard deviation

Table 2. Univariate and Multivariate Cox Proportional-Hazards Regression Analysis of the Anthracycline Cohort

Characteristics	Unadjusted HR	(95% CI)	p-value	Adjusted HR	(95% CI)	p-value
Anthracycline Dose						
≤ 240 mg/m^2	Reference	---		Reference	---	
> 240 mg/m^2	1.68	(0.76, 3.68)	0.17	1.72	(0.70, 4.19)	0.24
Additional Treatment						
None	Reference	---		Reference	---	
Bevacizumab	3.56	(1.55, 8.20)	0.0028*	4.70	(1.78, 12.44)	0.0018*
Trastuzumab	7.96	(4.81, 13.18)	<0.001*	10.51	(5.83, 18.93)	<0.001*
Radiotherapy						
None	Reference	---		Reference	---	
Left-Sided Only	0.19	(0.10, 0.37)	<0.001*	0.16	(0.07, 0.35)	<0.001*
Right-Sided Only	0.32	(0.18, 0.56)	<0.001*	0.30	(0.16, 0.56)	<0.001*
Age						
≤ 65	Reference	---		Reference	---	
> 65	1.71	(0.78, 3.75)	0.18	1.67	(0.58, 4.83)	0.34
BMI						
Normal	Reference	---		Reference	---	
Overweight	0.93	(0.53, 1.62)	0.79	0.77	(0.40, 1.48)	0.43
Obese	0.77	(0.42, 1.41)	0.39	0.96	(0.44, 2.08)	0.91
Hypertension	0.64	(0.36, 1.14)	0.13	0.85	(0.39, 1.86)	0.68
Diabetes	0.79	(0.32, 1.97)	0.62	0.58	(0.19, 1.78)	0.34
Dyslipidemia	1.18	(0.69, 2.02)	0.55	1.69	(0.87, 3.32)	0.12
Smoking History	1.42	(0.79, 2.56)	0.24	2.82	(1.39, 5.71)	0.0039*
Family History of Cardiac Disease	0.96	(0.60, 1.54)	0.87	1.13	(0.65, 1.98)	0.66
Liver Disease	0.98	(0.42, 2.26)	0.95	1.01	(0.36, 2.83)	0.98

* Statistically significant at the $p < 0.05$ level; HR = hazard ratio; CI = confidence interval; BMI = body mass index

Table 3. Univariate and Multivariate Cox Proportional-Hazards Regression Analysis of the Trastuzumab Cohort

Characteristics	Unadjusted HR	(95% CI)	p-value	Adjusted HR	(95% CI)	p-value
Anthracycline Dose						
0 mg/m^2	Reference	---		Reference	---	
≤ 240 mg/m^2	1.03	(0.60, 1.77)	0.92	1.29	(0.68, 2.46)	0.44
> 240 mg/m^2	2.24	(0.76, 6.55)	0.14	2.49	(0.59, 10.5)	0.21
Radiotherapy						
None	Reference	---		Reference	---	
Left-Sided Only	0.27	(0.13, 0.56)	<0.001*	0.23	(0.10, 0.52)	<0.001*
Right-Sided Only	0.49	(0.27, 0.91)	<0.001*	0.31	(0.15, 0.64)	0.0017*
Age						
≤ 65	Reference	---		Reference	---	
> 65	3.97	(1.99, 7.91)	<0.001*	2.31	(0.78, 6.80)	0.13
BMI						
Normal	Reference	---		Reference	---	
Overweight	0.84	(0.46, 1.54)	0.57	0.61	(0.29, 1.28)	0.19
Obese	0.95	(0.50, 1.82)	0.88	0.85	(0.35, 2.05)	0.71
Hypertension	1.12	(0.64, 1.98)	0.68	1.35	(0.55, 3.36)	0.51
Diabetes	0.59	(0.19, 1.89)	0.38	0.31	(0.07, 1.48)	0.14
Dyslipidemia	2.03	(1.17, 3.52)	0.011*	3.66	(1.80, 7.42)	<0.001*
Smoking History	1.69	(0.93, 3.10)	0.087	3.01	(1.42, 6.39)	0.0040*
Family History of Cardiac Disease	0.79	(0.47, 1.33)	0.37	0.85	(0.44, 1.62)	0.62
Liver Disease	1.49	(0.59, 3.73)	0.40	2.07	(0.63, 6.73)	0.23

* Statistically significant at the $p < 0.05$ level; HR = hazard ratio; CI = confidence interval; BMI = body mass index

Table 4. Baseline Imaging Parameters: Descriptive Summary of Anthracycline Cohort

	All Breast Cancer Patients Receiving Anthracycline	Anthracycline - Bevacizumab - Trastuzumab	Anthracycline + Bevacizumab	Anthracycline + Trastuzumab	p-value
Characteristic	**n = 496**	**n = 389**	**n = 30**	**n = 76**	
Peak Filling Rate (PFR) (EDV/sec)					
Normal (≥ 2.5)	427 (86.26)	336 (86.60)	27 (90.00)	64 (84.21)	0.75
Abnormal (< 2.5)	68 (13.74)	52 (13.40)	3 (10.00)	12 (15.79)	
Left Ventricular End-Diastolic Volume (LV EDV) (ml)					
Normal (< 140)	414 (83.47)	324 (83.29)	27 (90.00)	62 (81.58)	0.62
Abnormal (≥ 140)	82 (16.53)	65 (16.71)	3 (10.00)	14 (18.42)	
Left ventricular ejection fraction (LVEF) (%)					
Normal (≥ 50)	491 (98.99)	386 (99.23)	30 (100.00)	75 (98.68)	0.62
Abnormal (< 50)	5 (1.01)	3 (0.77)	0 (0.00)	1 (1.32)	

Note: Data are n (%) of persons for whom data are available. Percentages may not sum to one hundred due to rounding.

* Statistically significant at the $p < 0.05$ level

Table 5. Baseline Imaging Parameters: Univariate and Multivariate Cox Proportional-Hazards Regression Analysis of the Anthracycline Cohort

Baseline Imaging Parameter	Unadjusted HR	(95% CI)	p-value	Adjusted HR	(95% CI)	p-value
Peak Filling Rate (PFR) (EDV/sec)						
Normal (≥ 2.5)	Reference	---		Reference	---	
Abnormal (< 2.5)	0.78	(0.37, 1.64)	0.51	0.74	(0.29, 1.90)	0.53
Left Ventricular End-Diastolic Volume (LV EDV) (ml)						
Normal (< 140)	Reference	---		Reference	---	
Abnormal (≥ 140)	1.80	(1.05, 3.08)	0.032*	1.28	(0.63, 2.61)	0.50

* Statistically significant at the $p < 0.05$ level

HR = hazard ratio; CI = confidence interval

Adjusted for cumulative anthracycline dose, additional treatment, chest radiotherapy, age, BMI, smoking history, family history of cardiac disease, and comorbidities (hypertension, diabetes, dyslipidemia, and liver disease) in a multivariate Cox proportional-hazards model