ORIGINAL RESEARCH

Changes in Cardiovascular Biomarkers With Breast Cancer Therapy and Associations With Cardiac Dysfunction

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***Background*—**We examined the longitudinal associations between changes in cardiovascular biomarkers and cancer therapy–related cardiac dysfunction (CTRCD) in patients with breast cancer treated with cardotoxic cancer therapy.

***Methods and Results*—**Repeated measures of high-sensitivity cardiac troponin T (hs-cTnT), NT-proBNP (N-terminal pro-B-type natriuretic peptide), myeloperoxidase, placental growth factor, and growth differentiation factor 15 were assessed longitudinally in a prospective cohort of 323 patients treated with anthracyclines and/or trastuzumab followed over a maximum of 3.7 years with serial echocardiograms. CTRCD was defined as a ≥10% decline in left ventricular ejection fraction to a value <50%. Associations between changes in biomarkers and left ventricular ejection fraction were evaluated in repeated-measures linear regression models. Cox regression models assessed the associations between biomarkers and CTRCD. Early increases in all biomarkers occurred with anthracycline-based regimens. hs-cTnT levels >14 ng/L at anthracycline completion were associated with a 2-fold increased CTRCD risk (hazard ratio, 2.01; 95% CI, 1.00–4.06). There was a modest association between changes in NT-proBNP and left ventricular ejection fraction in the overall cohort; this was most pronounced with sequential anthracycline and trastuzumab (1.1% left ventricular ejection fraction decline [95% CI, −1.8 to −0.4] with each NT-proBNP doubling). Increases in NT-proBNP were also associated with CTRCD (hazard ratio per doubling, 1.56; 95% CI, 1.32–1.84). Increases in myeloperoxidase were associated with CTRCD in patients who received sequential anthracycline and trastuzumab (hazard ratio per doubling, 1.28; 95% CI, 1.04–1.58).

***Conclusions*—**Cardiovascular biomarkers may play an important role in CTRCD risk prediction in patients with breast cancer who receive cardiotoxic cancer therapy, particularly in those treated with sequential anthracycline and trastuzumab therapy.

***Clinical Trial Registration*—**URL: https://www.clinicaltrials.gov/. Unique identifier: NCT01173341. **(*J Am Heart Assoc*. 2020;9: e014708. DOI: 10.1161/JAHA.119.014708.)**

Key Words: biomarker • cardiomyopathy • cardio-oncology • cardiotoxicity

Breast cancer is the most common cancer in women. Although marked improvements in the detection and treatment of breast cancer have led to substantial gains in disease-specific survival, cardiovascular morbidity and mortality associated with commonly used cardiotoxic cancer therapies remain a significant concern. Declines in cardiac function with agents such as anthracyclines and trastuzumab can result in treatment interruptions and delays in the short term. In the long term, the risk of cardiovascular mortality may exceed that of recurrent cancer, particularly in older women.[1]

Within cardio-oncology, there has been a focus on the early detection of cancer therapy–related cardiac dysfunction

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Accompanying Data S1, Tables S1 through S7, and Figures S1 through S5 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.014708

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Clinical Perspective

What Is New?

- This is the largest prospective cohort study of patients with breast cancer treated with anthracyclines and/or trastuzumab with detailed cardiovascular phenotyping, including biomarkers, echocardiography, and clinical data, over an extended follow-up.
- High-sensitivity cardiac troponin T elevation is common following anthracycline therapy, and its assessment specifically at the completion of anthracyclines may be informative in cancer therapy–related cardiac dysfunction risk prediction.
- Changes in N-terminal pro-B-type natriuretic peptide show temporal associations with changes in imaging measures of cardiac dysfunction and predict the risk of cancer therapy–related cardiac dysfunction, particularly in patients treated with sequential anthracycline and trastuzumab therapy, and myeloperoxidase shows promise as a candidate novel biomarker of cardiotoxicity with anthracycline-based cancer therapy regimens.

What Are the Clinical Implications?

- Biomarkers can play an important role in cancer therapy–related cardiac dysfunction risk prediction in patients with breast cancer who receive cardiotoxic cancer therapy.
- The findings of the study motivate randomized controlled trials aimed at investigating whether biomarker-guided strategies are of utility in mitigating cardiovascular risk.

(CTRCD) to avoid treatment delays and interruptions and decrease long-term cardiovascular risk.[2,3] Current guidelines recommend routine cardiac surveillance in patients with breast cancer treated with anthracyclines and/or trastuzumab.[4–6] Because of widespread availability, echocardiographic assessment of left ventricular ejection fraction (LVEF) is a recommended monitoring strategy. However, LVEF assessment is limited in the sensitive detection of early, subclinical changes in cardiac function.

Cardiac biomarkers are promising tools for the early detection and prediction of CTRCD.[7–9] Some prior studies have suggested that elevations in cardiac troponins (cTns) are common in patients treated with anthracyclines, with or without trastuzumab, and that elevations predict the development of cardiac dysfunction.[10–19] Our own published data have supported an association between troponin concentrations at the completion of anthracyclines and CTRCD but no association with serial troponin assessment at postanthracycline time points.[16,17] However, the reproducibility of these findings and a clear characterization of the utility of troponin assessment for the prediction of CTRCD are lacking. In addition, unanswered questions pertaining to the potential predictive value of natriuretic peptides and several newer biomarkers including myeloperoxidase, placental growth factor (PIGF), and growth differentiation factor 15 (GDF-15) also exist.[16–21]

To better characterize the role of biomarkers in patients with breast cancer who receive potentially cardiotoxic therapies, we comprehensively examined the longitudinal associations between changes in multiple cardiovascular biomarkers, including high-sensitivity cTn T (hs-cTnT), NT-proBNP (N-terminal pro-B-type natriuretic peptide), myeloperoxidase, PIGF, and GDF-15, and changes in LVEF and the risk of CTRCD in 323 patients with breast cancer treated with anthracyclines and/or trastuzumab, and determined differences according to cancer therapy regimen. The specific study objectives were to: (1) characterize the changes in multiple cardiovascular biomarkers in patients with breast cancer treated with anthracyclines and/or trastuzumab; (2) evaluate the longitudinal associations between changes in individual biomarkers and LVEF changes; (3) evaluate the associations between changes in biomarkers and the risk of CTRCD; (4) explore whether these associations differ according to cancer therapy regimen; and (5) describe the potential predictive value of these biomarkers.

Methods

Study Population

The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure. The CCT (Cardiotoxicity of Cancer Therapy) study is a prospective cohort of patients with breast cancer from the Rena Rowan Breast Center of the Abramson Cancer Center at the University of Pennsylvania (Philadelphia, Pennsylvania). Patients aged 18 years and older with breast cancer who were treated with anthracycline- and/or trastuzumab-based regimens were included. Exclusion criteria included pregnancy or an inability or unwillingness to provide informed consent. Treatment regimen was determined by the treating oncologist and consisted of either doxorubicin (240 mg/m^2 divided into 4 cycles of 60 mg/m^2 each) and cyclophosphamide followed by paclitaxel (doxorubicin group), doxorubicin (240 mg/m^2 divided into 4 cycles of 60 mg/m^2 each) and cyclophosphamide followed by paclitaxel and trastuzumab (doxorubicin+trastuzumab group), or trastuzumab with docetaxel and either cyclophosphamide or carboplatin (trastuzumab group). The study was approved by the institutional review board of the University of Pennsylvania. All participants provided written informed consent. B.D. and B.K. had full access to

all of the data in the study and take responsibility for their integrity and the data analysis.

Study Procedures

Detailed clinical data were collected using standardized questionnaires at baseline (ie, before treatment initiation) and during prespecified follow-up visits, and were further verified by reviewing medical records. Blood samples were drawn at standardized time intervals according to cancer therapy regimen (Figure 1).[22] In the doxorubicin group, blood samples were collected at baseline, during doxorubicin treatment (≈1 month), at the completion of doxorubicin (≈2 months), at the completion of paclitaxel (≈4 months), and then annually. In the doxorubicin+trastuzumab group, blood samples were collected at baseline, during doxorubicin treatment (≈1 month), at the completion of doxorubicin (≈2 months), every 6 weeks during trastuzumab therapy, and then annually. The median (interquartile range) interval between the completion of doxorubicin and initiation of trastuzumab therapy was 14 (14–15) days. In the trastuzumab group, blood samples were collected at baseline, every 6 weeks during trastuzumab therapy, and then annually. Blood samples were processed and stored at −80°C until assay. In addition, in the doxorubicin group, transthoracic echocardiograms were performed at baseline, at the completion of paclitaxel (≈4 months), and then annually. In the doxorubicin+trastuzumab group, echocardiograms were performed at baseline, at the completion of doxorubicin (≈2 months), every 3 months during trastuzumab therapy, and then annually. In the trastuzumab group, echocardiograms were performed at baseline, every 3 months during trastuzumab therapy, and then annually. Two-dimensional images were acquired using Vivid 7, E9, or E95 machines (GE Healthcare) and digitally archived for post hoc analyses.

Biomarker Measurements

Four biomarkers including hs-cTnT, NT-proBNP, PlGF, and GDF-15 were assessed in all treatment groups. hs-cTnT was measured using the fourth-generation Elecsys TnT-hs assay on the Cobas platform (Roche Diagnostics). NT-proBNP was measured using the Elecsys NT-proBNP assay on the Cobas platform. PlGF was measured using the Elecsys PlGF immunoassay. GDF-15 was measured with the Elecsys GDF-15 immunoassay. Myeloperoxidase was measured only in the doxorubicin and doxorubicin+trastuzumab groups, given its purported biologic relevance to anthracyclines specifically, using an ELISA assay (Cleveland HeartLab Inc). Detailed assay information is provided in Data S1.

Echocardiography Quantitation and CTRCD Definition

Quantitative echocardiography was performed by a single blinded observer at the University of Pennsylvania Center for Quantitative Echocardiography (Philadelphia, PA) using the TomTec Imaging Systems platform. Left ventricular end-diastolic and end-systolic volumes were calculated using the Simpson's method of discs and were utilized to derive LVEF. In addition, longitudinal and circumferential strain were analyzed. Intraobserver coefficients of variation were 4.9%, 10.9%, and 9.4% for LVEF, longitudinal strain, and

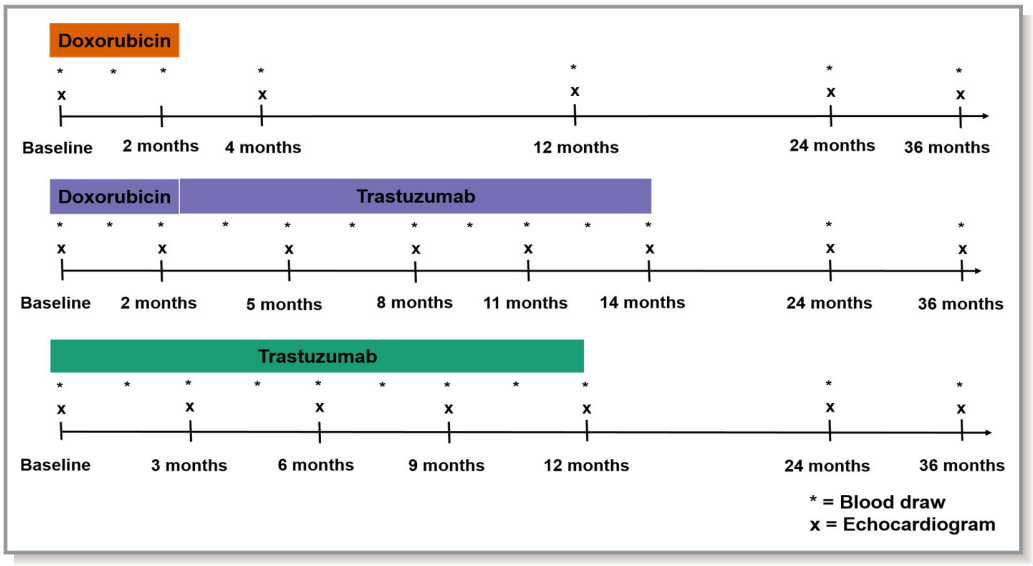

Figure 1. Study protocol. Timeline of blood draws and echocardiography assessment according to cancer therapy regimen.

circumferential strain, respectively.[23] CTRCD was defined as a ≥10% absolute decline in LVEF to a value <50%.[23,24]

Statistical Analysis

Baseline characteristics were summarized using proportions for categorical variables, and median (interquartile range) were presented for continuous variables. Given the skewed distributions, all biomarkers were log2 transformed. This also facilitated the comparison of the associations across all biomarkers. On the log2 scale, each 1-unit increase is equivalent to a doubling in biomarker levels.

To characterize the changes in biomarker levels according to individual treatment groups, mean estimated changes from baseline were plotted over time. Mean changes were determined using repeated-measures linear regression estimated via generalized estimating equations. Each model was adjusted for the baseline values of the biomarker under consideration and time since treatment initiation modeled nonparametrically using a cubic spline.

Contemporaneous associations between changes in biomarkers from baseline and changes in LVEF were determined using repeated-measures linear regression estimated via generalized estimating equations. Here, we used the independence working correlation structure. All models were adjusted for cancer therapy regimen, baseline LVEF, baseline biomarker value, time from treatment initiation (modeled nonparametrically using a cubic spline, whereby its effect was allowed to vary across treatment groups by including an interaction term), age, hypertension, smoking, and body mass index. Associations between changes from baseline in biomarker levels over time and subsequent changes in LVEF and longitudinal and circumferential strain were assessed similarly. Differences in the associations between changes in biomarkers and LVEF across the different treatment groups were evaluated by including biomarker-treatment interaction terms. Wald test was used to test statistical significance.

Associations between baseline biomarker values and time to CTRCD were assessed using Cox proportional hazards models. Longitudinal associations between repeated assessments of changes in biomarkers from baseline and time to CTRCD were determined using partly conditional survival models.[25] This approach provides a flexible, computationally efficient framework for longitudinal risk prediction and is well suited for analyses of relatively small sample sizes.[26] All models were adjusted for cancer therapy regimen, baseline LVEF, baseline biomarker value, age, hypertension, smoking, and body mass index. The proportional hazards assumption was evaluated for both the baseline biomarker and time-varying biomarker change variables by plotting Schoenfeld residuals over time. This assumption was not violated for any of the biomarker variables with the exception of baseline GDF-15 and change in NT-proBNP. Here, there was a notable decrease in the estimated residuals at late follow-up time points (Figures S1 and S2). We therefore conducted the analyses for baseline GDF-15 and change in NT-proBNP with follow-up time limited to 2 years, given the proportional hazards assumption was valid over this shorter follow-up period (Figure S3). To determine the incremental predictive value of biomarker changes to baseline clinical variables, the change in the concordance index was quantified.

To gain further insight into the predictive value of biomarkers, we performed additional analyses. We explored the association between elevated hs-cTnT (ie, >14 ng/L based on the sex-specific 99th percentile cutoff limit for the cTn assay used) at the completion of anthracyclines and subsequent CTRCD risk, given prior studies indicating the importance of troponin assessment at this time point in patients treated with anthracycline-based regimens.[14,16,18,19] We then quantified time-dependent sensitivity and specificity, and calculated the positive predictive value (PPV) and negative predictive value (NPV) at this threshold. In an exploratory analysis, we further examined these parameters at an hs-cTnT threshold of 5 ng/L (ie, limit of detection of the assay) as we postulated that this threshold could identify a subgroup with a low risk of CTRCD at the completion of anthracyclines. Furthermore, we explored test characteristics and the associated PPV and NPV at NT-proBNP thresholds of 125 ng/L, 150 ng/L, and 300 ng/L, based on previously published studies.[27–29]

Two-sided α levels <0.05 were considered statistically significant. All analyses were performed using R 3.4.0 (R Foundation for Statistical Computing).

Results

Study Population

In total, 323 patients had at least 1 biomarker measurement at baseline and 1 follow-up visit. The number of available measurements per biomarker is provided in Table S1. Baseline characteristics and biomarker values are summarized in Table 1. At baseline, the trastuzumab group tended to have higher median NT-proBNP levels, in particular, and patients in this group tended to be older, with higher systolic blood pressure, and a greater prevalence of hypertension compared with the other treatment groups.

Biomarker Trajectories According to Cancer Therapy Regimen

Changes in biomarkers over time according to the 3 cancer therapy regimens are presented in Figure 2A through 2E. In addition, changes in the combined doxorubicin groups as compared with the trastuzumab group are presented in

Table 1. Baseline Characteristics of the Overall Analysis Cohort and Stratified According to Cancer Therapy Regimen

Variable	Overall* (N=323)	Doxorubicin (n=199)	Trastuzumab (n=71)	Doxorubicin+ Trastuzumab (n=53)
Age, y	48 [41–57]	49 [41–56]	51 [44–58]	43 [38–54]
Race				
Black	81 (25.1)	58 (29.2)	7 (9.9)	16 (30.2)
White	224 (69.3)	130 (65.3)	60 (84.5)	34 (64.1)
Other/unknown	18 (5.6)	11 (5.5)	4 (5.6)	3 (5.7)
Breast cancer side				
Left	147 (45.6)	91 (45.8)	32 (45.7)	24 (45.3)
Right	157 (48.8)	97 (48.7)	37 (52.9)	23 (43.4)
Bilateral	18 (5.6)	11 (5.5)	1 (1.4)	6 (11.3)
Metastases or recurrence	4 (1.2)	1 (0.5)	3 (4.2)	0 (0)
Breast cancer stage				
1	69 (21.3)	27 (13.6)	32 (45.1)	10 (18.9)
2	178 (55.1)	123 (61.8)	28 (39.4)	27 (50.9)
3	69 (21.4)	47 (23.6)	6 (8.5)	16 (30.2)
4	7 (2.2)	2 (1.0)	5 (7.0)	0 (0)
Radiation therapy				
None	119 (37.0)	72 (36.4)	30 (42.2)	17 (32.1)
Left-sided	97 (30.1)	59 (29.8)	19 (26.8)	19 (35.8)
Right-sided	95 (29.5)	61 (30.8)	21 (29.6)	13 (24.5)
Bilateral	11 (3.4)	6 (3.0)	1 (1.4)	4 (7.6)
LVEF, %	53 [51–56]	53 [50–56]	53 [52–56]	54 [53–57]
Body mass index, kg/m^2				
<25	128 (39.7)	77 (38.7)	30 (42.2)	21 (39.6)
25 to 30	97 (30.0)	60 (30.1)	20 (28.2)	17 (32.1)
≥30	98 (30.3)	62 (31.2)	21 (29.6)	15 (28.3)
Systolic blood pressure, mm Hg	126 [116–135]	125 [115–135]	128 [117–140]	125 [116–134]
Diastolic blood pressure, mm Hg	74 [69–81]	75 [68–82]	73 [68–81]	74 [70–79]
Heart rate, beats per min	79 [72–89]	79 [72–89]	80 [72–90]	78 [74–87]
Current or prior smoking	128 (40.0)	83 (42.3)	25 (35.2)	20 (37.7)
History of diabetes mellitus	27 (8.4)	19 (9.5)	6 (8.5)	2 (3.8)
History of hypertension	99 (30.7)	60 (30.3)	28 (39.4)	11 (20.8)
History of hyperlipidemia or statin use	70 (21.7)	45 (22.6)	14 (20.0)	11 (20.8)
ACEI/ARB or β-blocker use	61 (18.9)	43 (21.6)	13 (18.3)	5 (9.4)
ACEI use	28 (8.7)	21 (10.6)	5 (7.0)	2 (3.8)
ARB use	22 (6.8)	13 (6.5)	8 (11.3)	1 (1.9)
β-Blocker use	22 (6.8)	17 (8.5)	3 (4.2)	2 (3.8)
hs-cTnT, ng/L	3 [3–4]	3 [3–4]	3 [3–5]	3 [3–4]
NT-proBNP, ng/L	68 [37–124]	58 [35–103]	140 [79–236]	60 [30–112]
GDF-15, ng/L	629 [509–892]	698 [538–897]	587 [425–945]	580 [519–707]

Continued

Table 1. Continued

Variable	Overall* (N=323)	Doxorubicin (n=199)	Trastuzumab (n=71)	Doxorubicin+ Trastuzumab (n=53)
PlGF, ng/L	13 [10–16]	13 [10–15]	15 [11–17]	13 [10–16]
Myeloperoxidase, pmol/L	307 [219–457]	303 [213–476]		325 [241–436]

ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; GDF-15, growth differentiation factor 15; hs-cTnT, high-sensitivity cardiac troponin T; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PlGF, placental growth factor.
*Patients with at least 1 available biomarker measurement at baseline and during at least 1 follow-up visit were included. Values are expressed as count (percentage) for categorical variables and median [interquartile range] for continuous variables. Myeloperoxidase was not measured in the trastuzumab group.

Figure S4. In the doxorubicin and doxorubicin+trastuzumab groups, hs-cTnT increased by ≈4-fold in the first 6 months and then declined to baseline levels. hs-cTnT levels remained low throughout follow-up in the trastuzumab group. On average, NT-proBNP demonstrated an early, modest elevation in the doxorubicin group but no substantial change with doxorubicin+trastuzumab. In the trastuzumab group, NT-proBNP significantly declined in the first 6 months; possibly in part related to the higher baseline levels in this treatment group.

For PlGF, early increases were observed in the doxorubicin, doxorubicin+trastuzumab, and to a lesser extent, in the trastuzumab groups, with a return to baseline levels over time. Early increases were followed by a decline to baseline in all treatment groups for GDF-15. Early increases were also observed for myeloperoxidase in the doxorubicin and doxorubicin+trastuzumab groups.

Associations Between Baseline and Changes in Biomarkers With LVEF Changes

This analysis was performed in 254 patients with quantitated echocardiograms at baseline and at least 1 follow-up visit. Baseline characteristics and biomarker values are summarized according to treatment group in this subset of patients in Table S2.

First, we determined the association between baseline biomarker levels and changes in LVEF. No significant associations were observed (Table S3). Next, we evaluated the contemporaneous associations between changes in biomarkers and changes in LVEF (ie, changes in biomarker levels and changes in LVEF over the same time interval). Changes in hs-cTnT and NT-proBNP from baseline had modest associations with LVEF changes at the same visit; LVEF declined by ≈0.6% per doubling of each of these biomarkers ($P<0.05$). No statistically significant associations were seen for the other biomarkers (Table 2).

Next, to gain insight into the ability of biomarkers to predict subsequent LVEF changes, we examined the association between preceding changes in biomarkers and LVEF changes at the subsequent visit. On average, there was a 0.7% (95% CI, −1.2 to −0.2) decline in LVEF at each subsequent visit per doubling of NT-proBNP ($P=0.004$), whereby the median time between biomarker and LVEF assessments was 2.1 months. No statistically significant associations were observed with the other biomarkers (Table 2). Associations between biomarker changes and circumferential strain were consistent with the LVEF findings; however, there were no significant associations between biomarker changes and longitudinal strain with the exception of myeloperoxidase (Table S4).

We also explored the associations between biomarker and LVEF changes according to treatment regimen, at both the contemporaneous and subsequent visits. There was a significant interaction between NT-proBNP and treatment regimen on the associations between biomarker and LVEF changes at the same ($P=0.030$) and subsequent visits ($P=0.017$). The most pronounced effect was observed in the doxorubicin+trastuzumab group; on average, LVEF declined by 1.1% to 1.3% per doubling of NT-proBNP from baseline for these associations (Figure 3 and Table S5).

Associations Between Baseline and Changes in Biomarker and CTRCD

Among the 254 patients with quantitated echocardiograms at baseline and during at least 1 follow-up visit, CTRCD occurred in 22 (14.2%), 9 (17.0%), and 18 (39.1%) patients in the doxorubicin, trastuzumab, and doxorubicin+trastuzumab groups, respectively, over a maximum period of 3.7 years of follow-up, consistent with previously published rates.[2,30,31] The majority had New York Heart Association class I heart failure (75.5%), while 24.5% developed New York Heart Association class II/III heart failure. The median (interquartile range) time to the development of CTRCD was 12 (5–22), 8 (5–9), and 8 (5–11) months from initiation of therapy in the doxorubicin, trastuzumab, and doxorubicin+trastuzumab groups, respectively.

First, we determined the associations between baseline biomarkers and CTRCD risk. A significant association was observed for myeloperoxidase. The hazard of CTRCD increased by 30% (hazard ratio, 1.30; 95% CI, 1.01–1.68)

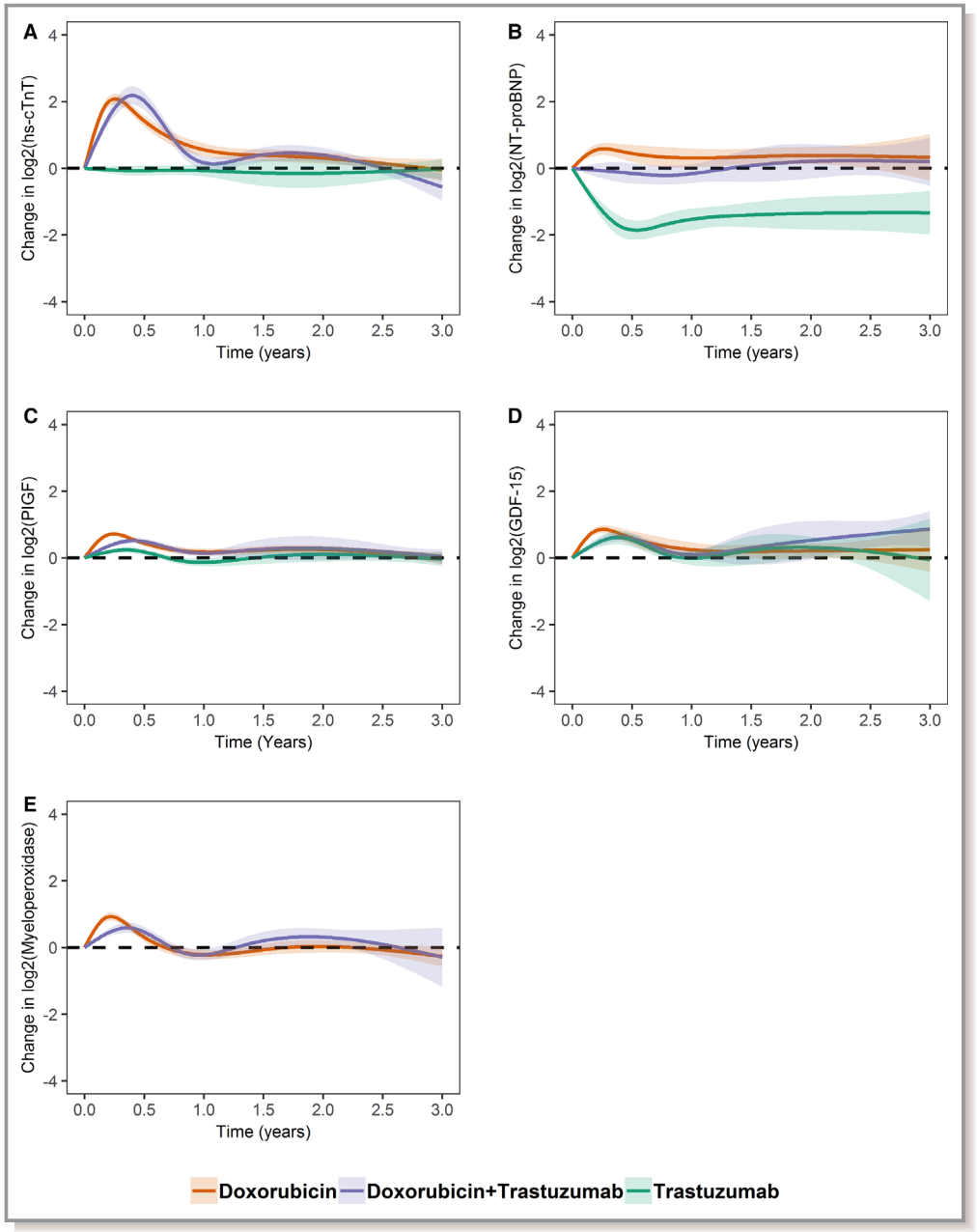

Figure 2. Mean estimated changes in biomarkers over time according to cancer therapy regimen. The solid line represents mean estimated changes over time and the width of the surrounding band represents the corresponding 95% CI. Biomarker levels were log2 transformed (a unit increment from baseline should be interpreted as doubling); (**A**) high-sensitivity cardiac troponin T (hs-cTnT), (**B**) NT-proBNP (N-terminal pro-B-type natriuretic peptide), (**C**) placental growth factor (PlGF), (**D**) growth differentiation factor 15 (GDF-15), and (**E**) myeloperoxidase.

per doubling in baseline myeloperoxidase value (P=0.041). No other statistically significant associations were observed (Table 3).

We next evaluated the associations between changes in biomarker levels from baseline and CTRCD risk in the overall cohort. We observed a significant association between changes in NT-proBNP and CTRCD. Each doubling in NT-proBNP from baseline was associated with a 56% increased risk (hazard ratio, 1.56; 95% CI, 1.32–1.84, P<0.001) over a maximum follow-up of 2 years (Table 3). Because of the limited number of CTRCD events within the different treatment groups, particularly in patients treated with trastuzumab alone, cancer therapy regimen–based stratified analysis was not performed. However, we specifically

Table 2. Associations Between Changes in Biomarker Levels and Changes in LVEF

	Contemporaneous		Subsequent Visit	
Biomarker	Beta (95% CI)	*P* Value	Beta (95% CI)	*P* Value
hs-cTnT	−0.6 (−1.1 to −0.1)	0.019	−0.3 (−0.8 to 0.2)	0.313
NT-proBNP	−0.6 (−1.1 to −0.2)	0.003	−0.7 (−1.2 to −0.2)	0.004
PlGF	0.6 (−0.1 to 1.4)	0.100	1.1 (0–2.2)	0.056
GDF-15	−0.2 (−0.9 to 0.5)	0.608	−0.3 (−1.4 to 0.7)	0.544
Myeloperoxidase	0.4 (−0.1 to 0.8)	0.105	0.4 (−0.2 to 0.9)	0.225

Biomarker levels were log2 transformed. Beta estimates represent the absolute change in the left ventricular ejection fraction (LVEF) for each doubling of biomarker levels from baseline to the same (contemporaneous) visit or the subsequent change in LVEF for each doubling in biomarker levels from baseline to the prior visit. Associations were adjusted for baseline variables including cancer therapy regimen, baseline LVEF, baseline biomarker levels, age, hypertension, smoking, body mass index, and time since treatment initiation (modeled nonparametrically using a cubic spline, its effect allowed to vary across treatment groups by including an interaction term). GDF-15 indicates growth differentiation factor 15; hs-cTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PlGF, placental growth factor.

evaluated the association between changes in myeloperoxidase and CTRCD in the doxorubicin+trastuzumab group to validate previous findings from our group that demonstrated predictive value of myeloperoxidase with this cancer therapy regimen.[16,17] Here, increases in myeloperoxidase were associated with increased CTRCD risk (hazard ratio per doubling 1.28; 95% CI, 1.04–1.57, *P*=0.019).

We then explored the incremental predictive value of biomarker changes to a baseline clinical model comprising cancer therapy regimen, LVEF, age, hypertension, body mass index, and smoking (Table S6). The addition of NT-proBNP change to this model improved the concordance index from 0.694 to 0.724. The incremental value of the other biomarkers was limited.

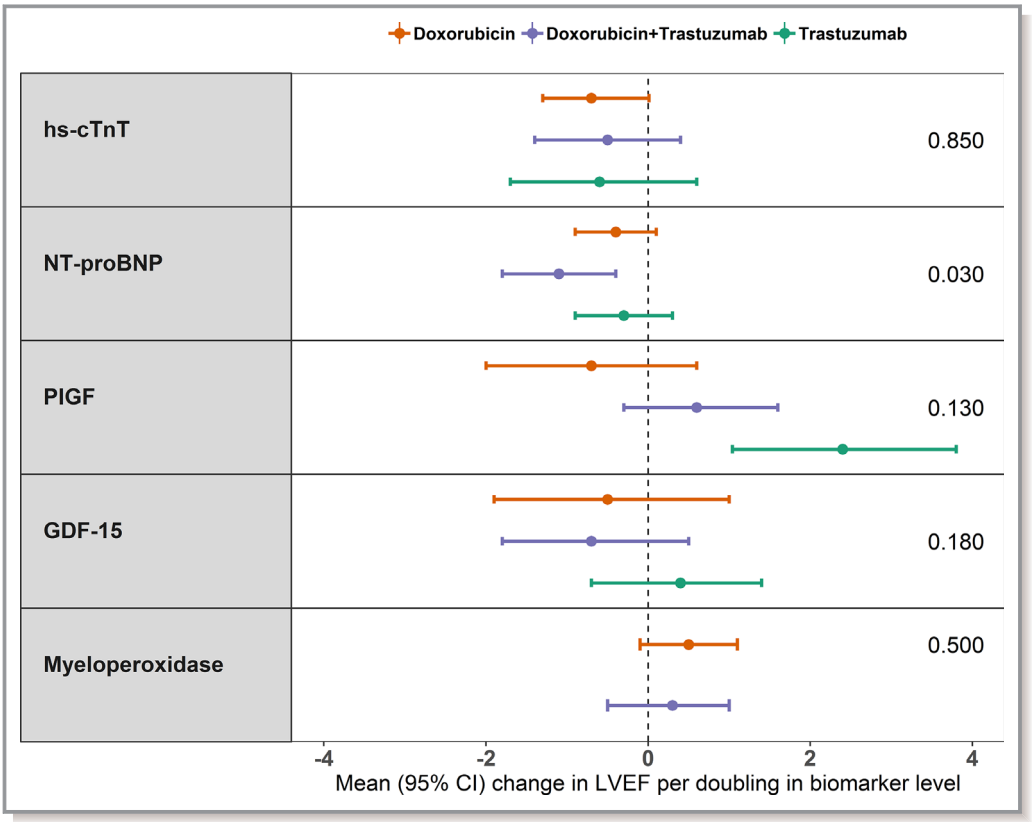

Figure 3. Associations between changes in biomarkers and changes in left ventricular ejection fraction (LVEF) according to cancer therapy regimen. Each point corresponds to mean absolute change in LVEF from baseline for each doubling of a biomarker from baseline to the same visit. The last column on the right side presents *P* values for interaction. GDF-15 indicates growth differentiation factor 15; hs-cTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PlGF, placental growth factor.

Table 3. Associations Between Baseline and Changes in Biomarker Levels and CTRCD (Defined as ≥10% Decline in LVEF to <50%)

	Baseline		Change From Baseline	
Biomarker	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
hs-cTnT	0.94 (0.55–1.59)	0.82	1.06 (0.87–1.31)	0.528
NT-proBNP*	1.00 (0.78–1.28)	>0.99	1.56 (1.32–1.84)	<0.001
PlGF	0.95 (0.53–1.70)	0.87	0.93 (0.87–1.31)	0.786
GDF-15*	1.12 (0.56–2.20)	0.75	0.96 (0.64–1.44)	0.834
Myeloperoxidase	1.30 (1.01–1.68)	0.041	1.10 (0.92–1.31)	0.300

Biomarker levels were log2 transformed. Hazard ratios (HRs) are for each doubling of biomarker level. Associations were adjusted for baseline biomarker levels and baseline variables including cancer therapy regimen, left ventricular ejection fraction (LVEF), age, hypertension, smoking, and body mass index. Associations between baseline biomarker levels and cancer therapy–related cardiac dysfunction (CTRCD) were modeled using Cox proportional hazards models, and associations between repeated assessments of change in biomarkers from baseline and CTRCD was modeled using partly conditional Cox models. hs-cTnT indicates high-sensitivity cardiac troponin T; PlGF, placental growth factor.
*For baseline growth differentiation factor 15 (GDF-15) and change in NT-proBNP (N-terminal pro-B-type natriuretic peptide) from baseline, the analyses were limited to the first 2 years of follow-up to address the violation of the proportional hazards assumption at late follow-up times (Figures S1 through S3).

Predictive Utility of hs-cTnT and NT-proBNP at the Completion of Anthracycline Therapy

Based on a priori hypothesis, we investigated the predictive value of hs-cTnT elevation at the time of completion of anthracycline chemotherapy among patients in the doxorubicin or doxorubicin+trastuzumab groups. Among 170 patients with available hs-cTnT at this time point, 71 (41.8%) had elevated hs-cTnT (ie, >14 ng/L). In multivariable analysis, elevated hs-cTnT at this time point was associated with a doubling of CTRCD risk (hazard ratio, 2.01; 95% CI, 1.00–4.06, *P*=0.052), consistent with previously published findings from our group and others.[13,16,18,19] At this threshold, hs-cTnT had a sensitivity and specificity of 60.3% and 62.5% for the prediction of CTRCD within 1 year after completion of anthracycline therapy; accompanying PPV and NPV were 26.5% and 87.5%, respectively (Table 4). Interestingly, an hs-cTnT level <5 ng/L at the completion of anthracycline therapy had 100% sensitivity and NPV for CTRCD at 1 year. However, <10% of the patients had hs-cTnT levels <5 ng/L at this time point (Table S7).

In terms of NT-proBNP elevations, 56 (33.1%) had levels >125 ng/L at completion of anthracyclines (Table S7). Elevations above this threshold at this time point had a sensitivity of 42.3% and NPV of 84.3% (Table 4). NT-proBNP concentrations at thresholds of 150 and 300 ng/L had higher specificity (ie, >85%) and PPV, but worse sensitivity and NPV (Table 4). The estimates of the PPV were 36.4% and 46.4% at 150 ng/L and 300 ng/L, respectively.

Discussion

To our knowledge, this is the largest prospective cohort study of patients with breast cancer treated with anthracyclines and/or trastuzumab with detailed cardiovascular phenotyping, including biomarkers, echocardiography, and clinical data, over an extended follow-up period of 3.7 years. This study comprehensively evaluated the potential predictive utility of both traditional and novel cardiovascular biomarkers at multiple time points during standard breast cancer systemic therapy. We demonstrate 4 key findings that advance our understanding of the role of biomarkers in cardio-oncology: (1) hs-cTnT elevations are common after anthracycline therapy; (2) elevated hs-cTnT specifically at the time of completion of anthracycline therapy may be predictive of subsequent CTRCD risk; (3) changes in NT-proBNP are significantly associated with changes in LVEF and CTRCD risk, and these associations are most pronounced in patients who receive sequential anthracycline and trastuzumab therapy; and (4) higher baseline levels of myeloperoxidase are associated with an increased risk of CTRCD, and changes in myeloperoxidase may have potential utility in predicting the

Table 4. Time-Dependent Sensitivity, Specificity, PPV, and NPV Estimates for hs-cTnT and NT-proBNP at the Completion of Anthracycline Therapy in the Doxorubicin or Doxorubicin+Trastuzumab Groups

Biomarker	Sensitivity, %	Specificity, %	PPV, %	NPV, %
hs-cTnT thresholds				
5 ng/L	100	10.2	20.0	100
14 ng/L	60.3	62.5	26.5	87.5
NT-proBNP thresholds				
125 ng/L	42.3	70.1	24.3	84.3
150 ng/L	37.7	85.1	36.4	85.8
300 ng/L	22.0	94.2	46.4	84.2

hs-cTnT indicates high-sensitivity cardiac troponin T; NPV, negative predictive value; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PPV, positive predictive value.

risk of CTRCD, particularly in patients treated with sequential anthracycline and trastuzumab therapy.

cTns are the most widely studied biomarkers in cardio-oncology. In early published work, cTnI elevation was reported in up to a third of patients treated with high-dose chemotherapy, and the pattern of release predicted the risk for cardiac events.[10,11] Recent studies utilizing high-sensitivity assays indicate that cTn elevation is even more common in patients treated with anthracycline-based regimens. However, the association between serial changes in cTn concentrations and the development of CTRCD have been less consistent.[10–17,32]

Our findings indicate that levels of hs-cTnT, on average, increase by nearly 4-fold early after the initiation of therapy, thereby suggesting that anthracyclines inflict cardiac injury broadly. However, changes in hs-cTnT levels were only modestly associated with changes in LVEF and circumferential strain. In addition, neither baseline values nor repeated assessment of changes in hs-cTnT were consistently associated with the development of CTRCD. Therefore, overall, our findings do not support the routine serial evaluation of hs-cTnT in patients with breast cancer treated with anthracyclines with or without trastuzumab for the prediction of systolic dysfunction. However, our data do suggest 2 important potential applications for hs-cTnT in this population, specifically at the time point of anthracycline completion. Here, hs-cTnT levels >14 ng/L were associated with a >2-fold increased risk of CTRCD, and this threshold had a PPV of 26.5% for the prediction of CTRCD at 1 year following the completion of therapy (which is 8% greater than the incidence of CTRCD within the subgroup of patients who received anthracyclines). This suggests that hs-cTnT elevation at the time of completion of anthracycline therapy might be considered as a possible enrichment strategy for defining a high-risk patient subpopulation. Another important observation was that none of the patients with hs-cTnT levels <5 ng/L after the completion of anthracycline therapy developed CTRCD over the subsequent year of follow-up (ie, 100% NPV). Although further validation is needed, this finding would suggest that hs-cTnT levels below the assay limit of detection at this time point might be considered as a possible strategy for identifying a low-risk subpopulation of patients. It is worth noting that our results do not exclude a role for hs-cTnT as a surrogate measure of subclinical injury with anthracycline-based regimens in the clinical trial setting.[32–37] Finally, our study further clarifies the role of hs-cTnT testing in patients treated with trastuzumab only. Our findings provide definitive evidence that, on average, hs-cTnT elevations are uncommon with trastuzumab therapy alone.[38]

Natriuretic peptides are also widely studied in patients treated with cardiotoxic cancer therapy. However, the predictive value of these biomarkers has not been consistent.[16–21] Prior studies mainly focused on the changes in natriuretic peptides during a relatively short follow-up time, mostly restricted to the time period during cancer therapy. Our study systematically examined the temporal associations between repeated assessments of changes in NT-proBNP and changes in imaging measures of cardiac function and CTRCD over an extended follow-up period. We observed consistent associations between increases in NT-proBNP and LVEF declines, which were particularly notable in the sequential anthracycline and trastuzumab group. Preceding increases in NT-proBNP were also associated with subsequent LVEF decline and worsening in circumferential strain, suggestive of a temporal association with these 2 measures of cardiac function. Importantly, the addition of NT-proBNP provided incremental value to baseline clinical risk factors for the prediction of CTRCD. Based on these results, we propose that routine serial assessment of NT-proBNP has the greatest potential utility in the cardiac surveillance of patients with breast cancer, particularly in those treated with sequential anthracycline and trastuzumab therapy. In exploratory analysis, we also observed that NT-proBNP concentrations at thresholds of 150 ng/L or 300 ng/L can identify subpopulations with increased subsequent CTRCD risk with high specificity and PPV that is 2- to 3-fold the incidence of CTRCD specifically in patients treated with the anthracycline-based cancer therapy regimen. Future studies are needed to validate these thresholds and further define their clinical utility in the setting of cardio-oncology.[39]

Among the newer biomarkers, myeloperoxidase has demonstrated promise as a biomarker of cardiac dysfunction in anthracycline-based treatment groups. As oxidative stress is a central mechanism in anthracycline-induced cardiotoxicity, myeloperoxidase is a mechanistically plausible marker of cardiotoxicity in patients treated with anthracyclines.[22,40,41] Prior small studies from our group showed that increased levels of myeloperoxidase during the course of therapy are associated with increased risk of cardiotoxicity with sequential anthracycline and trastuzumab therapy.[16,17] In line with these findings, our study showed that increases in myeloperoxidase, as well as baseline values, are associated with an increased risk of CTRCD with this cancer therapy regimen. These results would indicate that myeloperoxidase may be a candidate biomarker for the prediction of cardiotoxicity, particularly in patients treated with sequential anthracycline and trastuzumab therapy. However, further validation in larger studies is needed before it can be considered for clinical use as a marker of cardiotoxicity.

Modest early elevations in PlGF and GDF-15 were also observed with both anthracycline-based regimens and trastuzumab alone, suggesting that these agents might result in other pathophysiologic changes related to inflammation

and angiogenesis. However, neither PIGF nor GDF-15 showed significant associations with changes in LVEF and strain or CTRCD. Our findings do not support the role of PIGF and GDF-15 as predictive biomarkers of anthracycline and/or trastuzumab cardiotoxicity. This may appear to be in contrast with our previously published results;[17] however, we note that in the prior study, we studied the temporal changes in 8 biomarkers measured at 3-month intervals during doxorubicin and trastuzumab therapy, for a maximum follow-up time of 15 months. We sought to define both the concurrent and associations with subsequent cardiotoxicity and evaluated biomarkers over the entire course of follow-up and allowed for recurrent events (in contrast to the first event). Moreover, in prior studies, cardiotoxicity was defined by the Cardiac Review and Evaluation Committee criteria.[42] Thus, our exposures and outcomes were both defined and analyzed differently.

Study Strengths

Our study expands on prior work in this field and provides important findings that further clarify the role of biomarkers in the prediction of cardiac dysfunction in patients with breast cancer exposed to cardiotoxic cancer therapy. The current study has several strengths, particularly related to study design and methodology, which make it unique from previous studies. First, the prospective, longitudinal cohort study design coupled with the relatively large sample size and longer follow-up duration extending well beyond the time of completion of cancer therapy is an important strength as compared with previous studies. Moreover, biomarkers were evaluated at multiple prespecified time points, including both during and after completion of cancer therapy. Previous studies have mainly focused on changes in biomarker levels during the course of cancer therapy. Third, our study was composed of a nonclinical trial population, which enhances the generalizability of our findings. Fourth, the current study is the first to systematically characterize differences in the pattern of biomarker changes and their associations with changes in measures of cardiac dysfunction across different cancer therapy regimens. Fifth, the utilization of more robust statistical tools for the analysis of longitudinal data including generalized estimating equations and partly conditional survival models is another novel aspect of the study.

Our results motivate additional research. An important next step should be establishing validated biomarker thresholds that can be used as benchmarks to facilitate the detection of cardiotoxicity and prediction of subsequent risk, particularly for hs-cTnT and NT-proBNP. This can enhance the clinical applicability of biomarker information in daily clinical practice. Moreover, dynamic risk prediction models combining clinical data with serial measurements of multiple biomarkers could be developed in larger data sets and externally validated. More importantly, randomized controlled trials are needed to determine whether biomarker-guided cardioprotective treatment strategies are of utility in mitigating cardiovascular risk. Lastly, the value of serial assessment of biomarker changes appears to be primarily in patients treated with sequential anthracycline and trastuzumab therapy. This highlights a significant need for biomarker discovery research to identify novel mechanistic biomarkers of cardiotoxicity with anthracyclines or trastuzumab alone.[22]

Study Limitations

Our study has limitations that warrant consideration. The hs-cTn assay used is relatively less sensitive than some even newer methods. It thus remains possible that our results might differ if other, more highly sensitive methods were used for cTn assessment. As a result of the relatively small size of the treatment groups and number of CTRCD events, we could not reliably evaluate biomarker-treatment regimen interactions for the CTRCD outcome, and these analyses were limited to LVEF change alone as the outcome measure. We also did not adjust for multiple comparisons. As such, the association models should be interpreted in that context. Another limitation is that myeloperoxidase was not measured in the trastuzumab group. Importantly, our findings may not be generalizable to other cancer populations receiving additional cardiotoxic therapies and with different cardiovascular risk profiles. As it relates to NT-proBNP, we did observe decreases during trastuzumab therapy, particularly in the trastuzumab group. The reasoning behind this is not clear at this stage, although this is unlikely to have impacted any associations with CTRCD. We explored whether this may in part be attributable to confounding by variables such as age and hypertension, as the trastuzumab group tended to be older and more hypertensive with higher baseline NT-proBNP levels. However, adjustment for potential confounders did not significantly alter the pattern of change over time observed for NT-proBNP with this treatment regimen (Figure S5). We cannot exclude the possibility of an assay interaction between trastuzumab and NT-proBNP, but further work is needed to clarify this and should be a topic of future basic studies.

Conclusions

Our study demonstrates that hs-cTnT elevations are common in patients with breast cancer treated with anthracycline-based cancer therapy regimens, and assessment at the completion of anthracycline therapy may have clinical utility in cardiotoxicity risk prediction. We determined a consistent

temporal association between changes in NT-proBNP and cardiotoxicity, which was particularly notable in the sequential anthracycline and trastuzumab group. The study also provides incremental data to support the potential role for myeloperoxidase as a candidate novel biomarker of cardiotoxicity in patients treated with sequential anthracycline and trastuzumab therapy. Altogether, our findings inform clinically relevant strategies to mitigate cardiovascular risk in patients with breast cancer who receive cardiotoxic therapies.

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Biomarker measurements

Four biomarkers including high sensitivity cardiac troponin T (hs-cTnT), N-terminal pro-B-type natriuretic peptide (NT-proBNP), placental growth factor (PIGF) and growth differentiation factor 15 (GDF-15) were assessed in all treatment groups. hs-cTnT was measured using the 4th generation Elecsys® TnT-hs assay on Cobas® platform (Roche Diagnostics, Manheim, Germany) which has a coefficient of variation (CV) of <10% at the 99th percentile upper reference limit of 14 ng/L, a limit of detection of 5 ng/L and limit of blank of 3 ng/L. NT-proBNP was measured using the Elecsys® NT-proBNP assay on Cobas® platform, which has a CV of 2.9 to 6.1% and measurement range of 5-35,000 ng/L. PIGF was measured using the Elecsys® PIGF immunoassay with a CV of <5% and measurement range of 3-10,000 ng/L. GDF-15 was measured with the Elecsys® GDF-15 immunoassay with a measurement range is 400-20,000 ng/L and CV <10%. GDF-15 reagents were available for a slightly smaller subset of individuals. In addition, myeloperoxidase (MPO) was measured in the Doxorubicin and Doxorubicin+Trastuzumab treatment groups using an ELISA assay (Cleveland HeartLab Inc, Cleveland Ohio) on a Roche Cobas® 6000 platform with a c501 module.

Table S1. **Number of available biomarker measurements in the analysis patient population.**

Biomarker	Median (IQR) number of measurements per patient	Total number of measurements within different intervals from the time of cancer therapy initiation			
		<6 months	6-12 months	12-24 months	24-36 months
hs-cTnT	5 [4 - 8]	1238	393	194	115
NT-proBNP	5 [4 - 8]	1231	392	194	115
PIGF	5 [4 - 8]	1219	391	194	115
GDF-15	3 [0 - 5]	753	247	99	38
MPO	5 [4 - 6]	974	233	174	91
GDF-15 was measured in a smaller subset of patients given limitations in the number of reagents; MPO was analyzed only in the Doxorubicin and Doxorubicin+Trastuzumab groups					

Table S2. Baseline characteristics stratified according to cancer therapy regimen in the subset of patients with quantitated echocardiograms at baseline and during at least one follow-up visit (N=254).

Variable	Doxorubicin (N=155)	Trastuzumab (N=53)	Doxorubicin+ Trastuzumab (N=46)
Age (years)	49 [41 - 57]	52 [45 - 58]	43 [38 - 53]
Race, %(N))			
Black	41 (26.5)	6 (11.3)	14 (30.4)
Caucasian/other/unknown	114 (73.5)	47 (88.7)	32 (69.6)
Breast cancer side, % (N)			
Left	71 (45.8)	21 (39.6)	21 (45.7)
Right	79 (51.0)	31 (58.5)	19 (41.3)
Bilateral	5 (3.2)	1 (1.9)	6 (13.0)
Metastases or recurrence, % (N)	1 (0.6)	3 (5.7)	0 (0)
Breast cancer stage, % (N)			
Stage 1	21 (13.5)	22 (41.5)	9 (19.6)
Stage 2	96 (61.9)	24 (45.3)	23 (50)
Stage 3	37 (23.9)	4 (7.5)	14 (30.4)
Stage 4	1 (0.6)	3 (5.7)	0 (0)
Radiation therapy, % (N)			
None	56 (36.4)	23 (43.4)	15 (32.6)
Left-sided	46 (29.9)	13 (24.5)	16 (34.8)
Right-sided	48 (31.2)	16 (30.2)	11 (23.9)
Bilateral	4 (2.6)	1 (1.9)	4 (8.7)

Left ventricular ejection fraction (%)	53 [51 - 56]	53 [52 - 56]	54 [53 - 57]
Body mass index (kg/m^2)			
<25	60 (38.7)	23 (43.4)	17 (37)
25-30	51 (32.9)	17 (32.1)	16 (34.8)
≥30	44 (28.4)	13 (24.5)	13 (28.3)
Systolic blood pressure (mmHg)	124 [116 - 133]	127 [116 - 136]	124 [116 - 134]
Diastolic blood pressure (mmHg)	74 [69 - 81]	74 [69 - 81]	73 [70 - 79]
Heart rate (bpm)	80 [72 - 89]	78 [72- 90]	79 [73 - 89]
Current or past Smoking , %(N)	64 (41.8)	18 (34)	15 (32.6)
History of diabetes mellitus, %(N)	14 (9)	4 (7.5)	2 (4.4)
History of hypertension, %(N)	46 (29.9)	21 (39.6)	10 (21.7)
History of hyperlipidemia or statin use, %(N)	35 (22.6)	8 (15.4)	11 (23.9)
Hyperlipidemia, %(N)	34 (21.9)	8 (15.4)	11 (23.9)
Statin use, %(N)	15 (9.7)	6 (11.3)	4 (8.7)
ACEI/ARB or beta-blocker, %(N)	33 (21.3)	10 (18.9)	5 (10.9)
ACEI, %(N)	17 (11)	5 (9.4)	2 (4.3)
ARB, %(N)	8 (5.2)	5 (9.4)	1 (2.2)
Beta-blocker, %(N)	11 (7.1)	3 (5.7)	2 (4.3)
hs-cTnT (pg/mL)	3 [3 - 4]	3 [3 - 5]	3 [3 -4]
NT-proBNP (pg/mL)	61 [37 - 100]	137 [79 240]	62 [31 - 111]
GDF-15 (pg/mL)	704 [532 - 908]	585 [435 - 902]	599 [523 - 722]
PIGF (pg/mL)	13 [10 - 16]	14 [10 - 17]	13 [10 - 16]
MPO (pmol/L)	308 [212 - 499]		344 [247 - 446]
Count (%) is presented for categorical variables; median [interquartile range (IQR)] is presented for continuous variables; ACEI=angiotensin-converting enzyme inhibitor,			

ARB=angiotensin receptor blocker; GDF-15=Growth differentiation factor 15; hs-cTnT= High sensitivity cardiac troponin T; MPO=Myeloperoxidase; NT-proBNP= N-terminal pro-B-type natriuretic peptide; PIGF=Placental growth factor; MPO was not measured in the Trastuzumab group

Table S3. Associations between baseline biomarker levels and changes in LVEF.

Biomarker	Change in LVEF	
	Beta (95% CI)	**P-value**
hs-cTnT	0.1 (-0.5, 0.8)	0.73
NT-proBNP	0.2 (-0.3, 0.7)	0.38
PIGF	0.0 (-0.8, 0.9)	0.93
GDF-15	0.2 (-0.8, 1.1)	0.76
MPO	-0.2 (-0.6, 0.2)	0.39
Biomarker levels were log2 transformed; Beta estimates should be interpreted per doubling of baseline biomarker levels; Associations were adjusted for baseline variables including LVEF, age, hypertension, smoking, BMI and time since treatment initiation (modeled non-parametrically using cubic spline and its effect allowed to vary across treatment groups by including an interaction term)		

Table S4. Associations between changes in biomarker levels and changes in circumferential and longitudinal strain.

Biomarker	Circumferential strain (%)				Longitudinal strain (%)			
	Contemporaneous		Subsequent Visit		Contemporaneous		Subsequent Visit	
	Beta (95% CI)	P-value	Beta (95% CI)	P-value	Beta (95% CI)	P-value	Beta (95% CI)	P- value
hs-cTnT	0.5 (0.1,1.03	0.023	0.4 (0.1,0.8)	0.026	0.1 (-0.1,0.4)	0.267	0.2 (0,0.4)	0.101
NT-proBNP	0.4 (-0.1,0.8)	0.109	0.6 (0.2,1.0)	0.006	0 (-0.3,0.2)	0.862	0.1 (-0.2,0.4)	0.575
PIGF	0.1 (-0.5,0.8)	0.665	0.3 (-0.8,1.5)	0.559	-0.3 (-1.0,0.3)	0.320	0 (-0.6,0.7)	0.950
GDF-15	0.2 (-0.4,0.9)	0.477	0.8 (-0.1,1.6)	0.0687	0.5 (-0.1,1.2)	0.089	0.1 (-0.4,0.6)	0.663
MPO	0.4 (0.1,0.8)	0.011	0.4 (-0.2,0.9)	0.170	0.1 (-0.2,0.4)	0.570	0.4 (0.1,0.6)	0.007

Biomarker levels were log2 transformed; Beta estimates represent the absolute change in the outcome under consideration for each doubling of biomarker levels from baseline to the same (contemporaneous) visit; or the subsequent change in the outcome for each doubling in biomarker value from baseline to the prior visit. Associations were adjusted for baseline variables including cancer therapy regimen, baseline levels of the outcome under consideration, baseline biomarker levels, age, hypertension, smoking, BMI and time since treatment initiation (modeled non-parametrically using a cubic spline, its effect allowed to vary across treatment groups by including an interaction term); GDF-15=Growth differentiation factor 15; hs-cTnT= High sensitivity cardiac troponin T; MPO=Myeloperoxidase; NT-proBNP= N-terminal pro-B-type natriuretic peptide; PIGF=Placental growth factor.

Table S5. Associations between changes in biomarker levels from baseline to a visit and change in LVEF at the subsequent visit; Interactions with cancer therapy regimen.

Biomarker	Doxorubicin	Trastuzumab	Doxorubicin+Trastuzumab	P-interaction
	Mean change in LVEF (95% CI)	Mean change in LVEF (95% CI)	Mean change in LVEF (95% CI)	
hs-cTnT	-0.3 (-0.9, 0.2)	0.7 (-0.6, 1.9)	-0.6 (-1.6, 0.5)	0.550
NT-proBNP	-0.6 (-1.3, 0.1)	-0.2 (-0.8, 0.4)	-1.3 (-2.0, -0.6)	0.017
PIGF	-0.3 (-1.6, 1.0)	2.6 (0.7, 4.4)	1.7 (-0.6, 4.0)	0.270
GDF-15	-0.7 (-2.3, 0.9)	-0.5 (-2.4, 1.4)	0.8 (-0.7, 2.3)	0.110
MPO	0.6 (-0.1, 1.2)	-	-0.2 (-1.4, 1.0)	0.290
Biomarker levels were log2 transformed; Beta estimates should be interpreted per doubling in biomarker levels from baseline to prior visit; Associations were adjusted for baseline biomarker levels and baseline variables including LVEF, age, hypertension, smoking, BMI and time since treatment initiation (modeled non-parametrically using cubic spline)				

Table S6. Incremental predictive value of biomarker change variables when added to a baseline clinical model.

Biomarker	Clinical model + biomarker	Absolute Change in Concordance index
hs-cTnT	0.699	0.005
NT-proBNP	0.724	0.030
MPO	0.692	0.008
NT-proBNP + hs-cTnT	0.724	0.030
All biomarker values were log2 transformed; The clinical model included cancer therapy regimen, LVEF, age, hypertension, body mass index (BMI) and smoking *The clinical model had a concordance index of 0.694 in the overall group, and 0.684 in the combined Doxorubicin and Doxorubicin+Trastuzumab groups (Note: MPO is only available in these treatment groups)		

Table S7. Proportion of patients with elevated hs-cTnT and NT-proBNP at the completion of anthracycline therapy.

Biomarkers	**N (%)**
hs-cTnT > 5 ng/L	156 (91.8)
hs-cTnT >14 ng/L	71 (41.8)
NT-proBNP >125 ng/L	56 (33.1)
NT-proBNP >150 ng/L	40 (23.7)
NT-proBNP >300 ng/L	13 (7.7)
For the Doxorubicin+ Trastuzumab group, patients treated with anthracyclines then trastuzumab were included; A total of 170 and 169 measurements were available at the time of completion of doxorubicin therapy for hs-cTnT and NT-proBNP, respectively.	

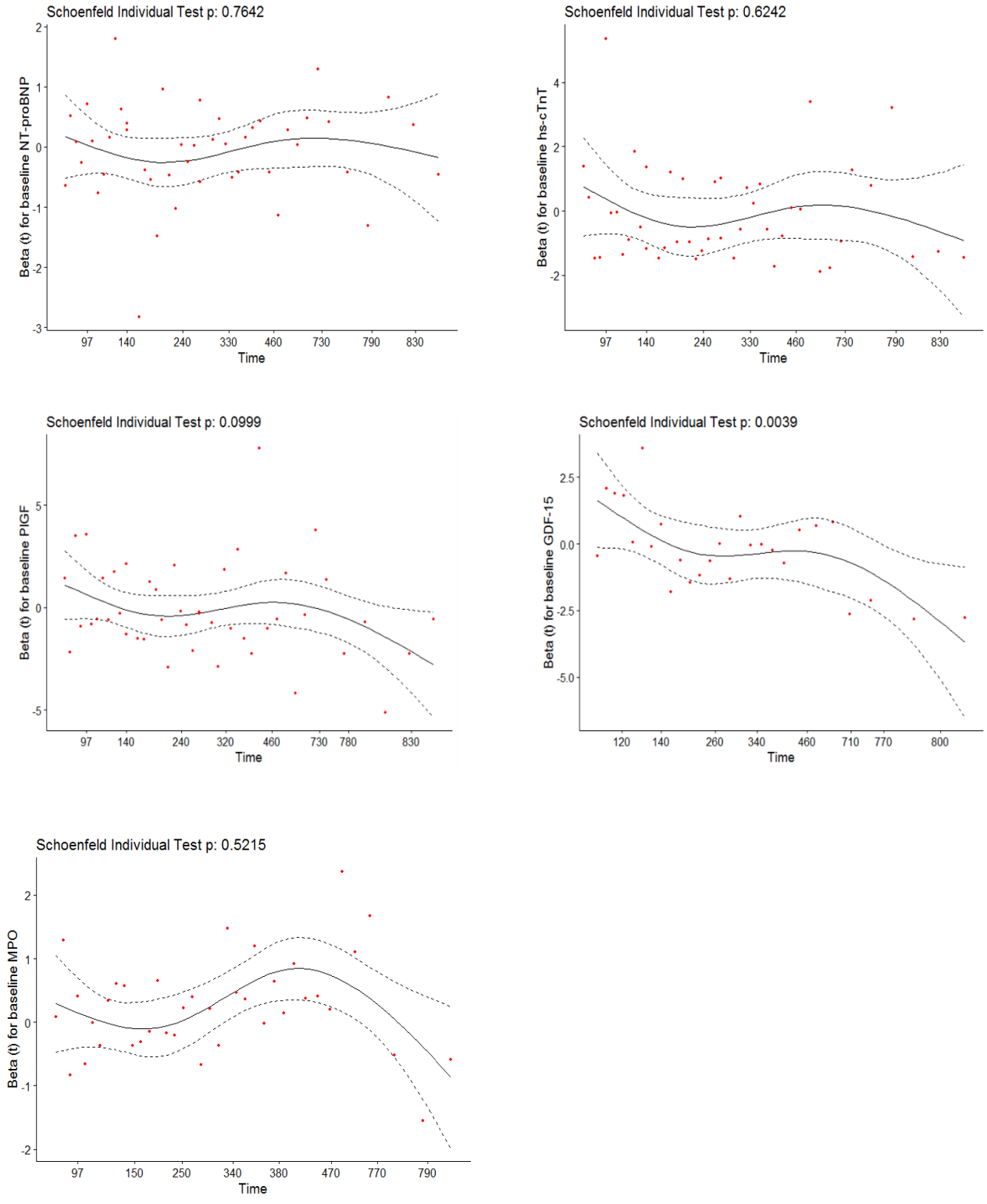

Figure S1. Plots of Schoenfeld residuals against time for the associations between baseline biomarkers and time to cancer therapy-related cardiac dysfunction.

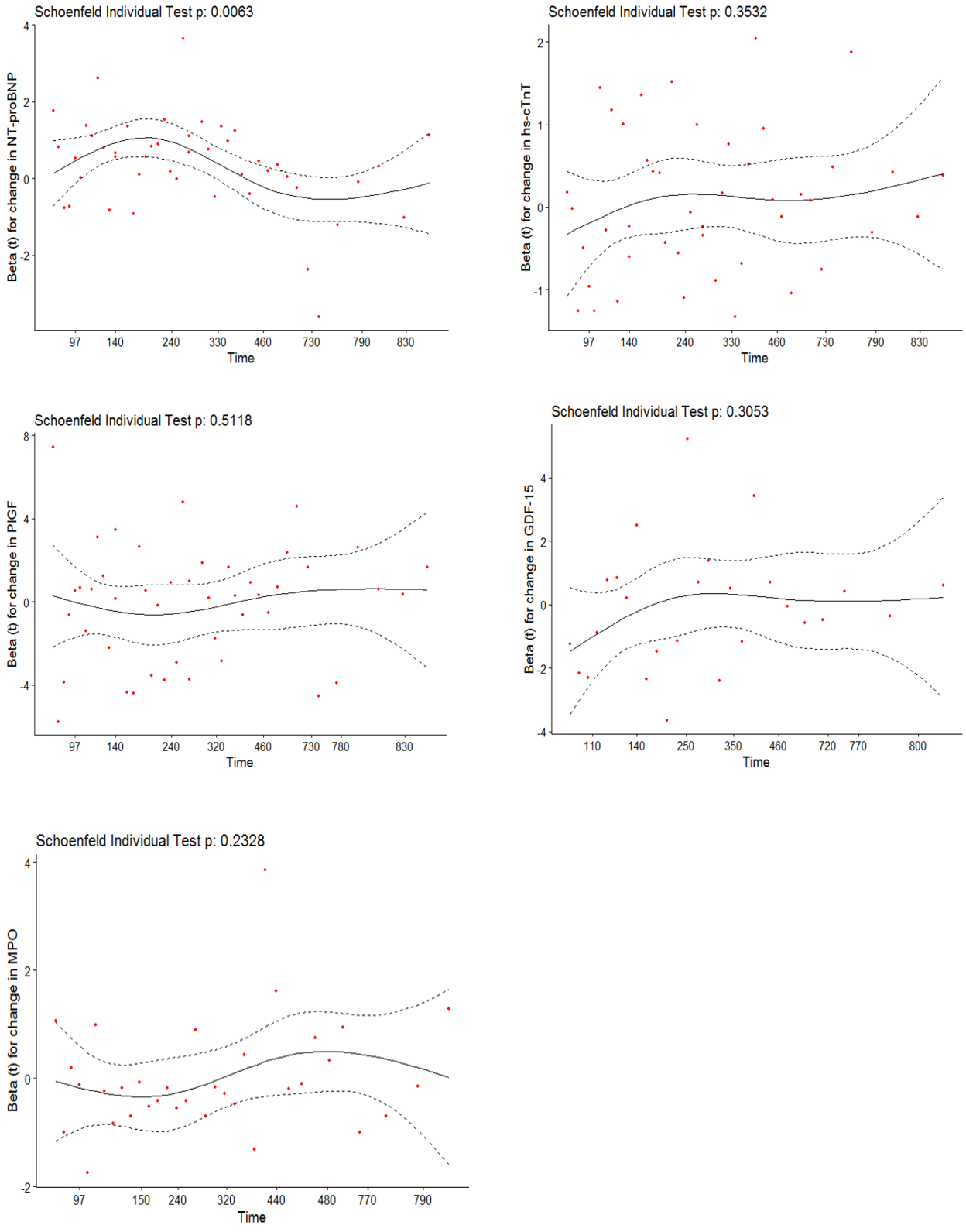

Figure S2. Plots of Schoenfeld residuals against time for the associations between changes in biomarkers and time to cancer therapy-related cardiac dysfunction.

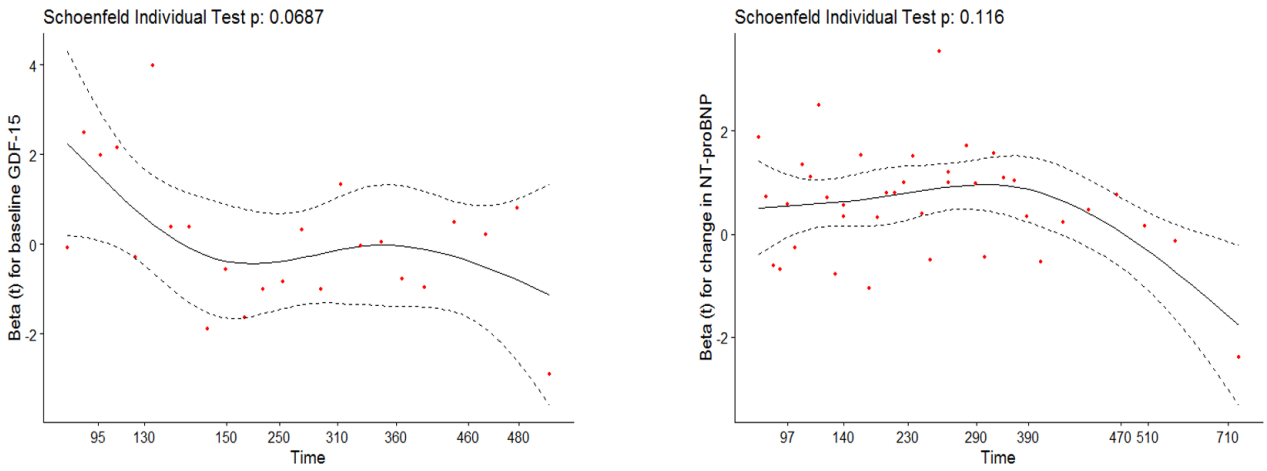

Figure S3. Plots of Schoenfeld residuals against time for the associations between baseline GDF-15 and change in NT-proBNP, and time to cancer therapy-related cardiac dysfunction limiting maximum follow-up time to 2 years.

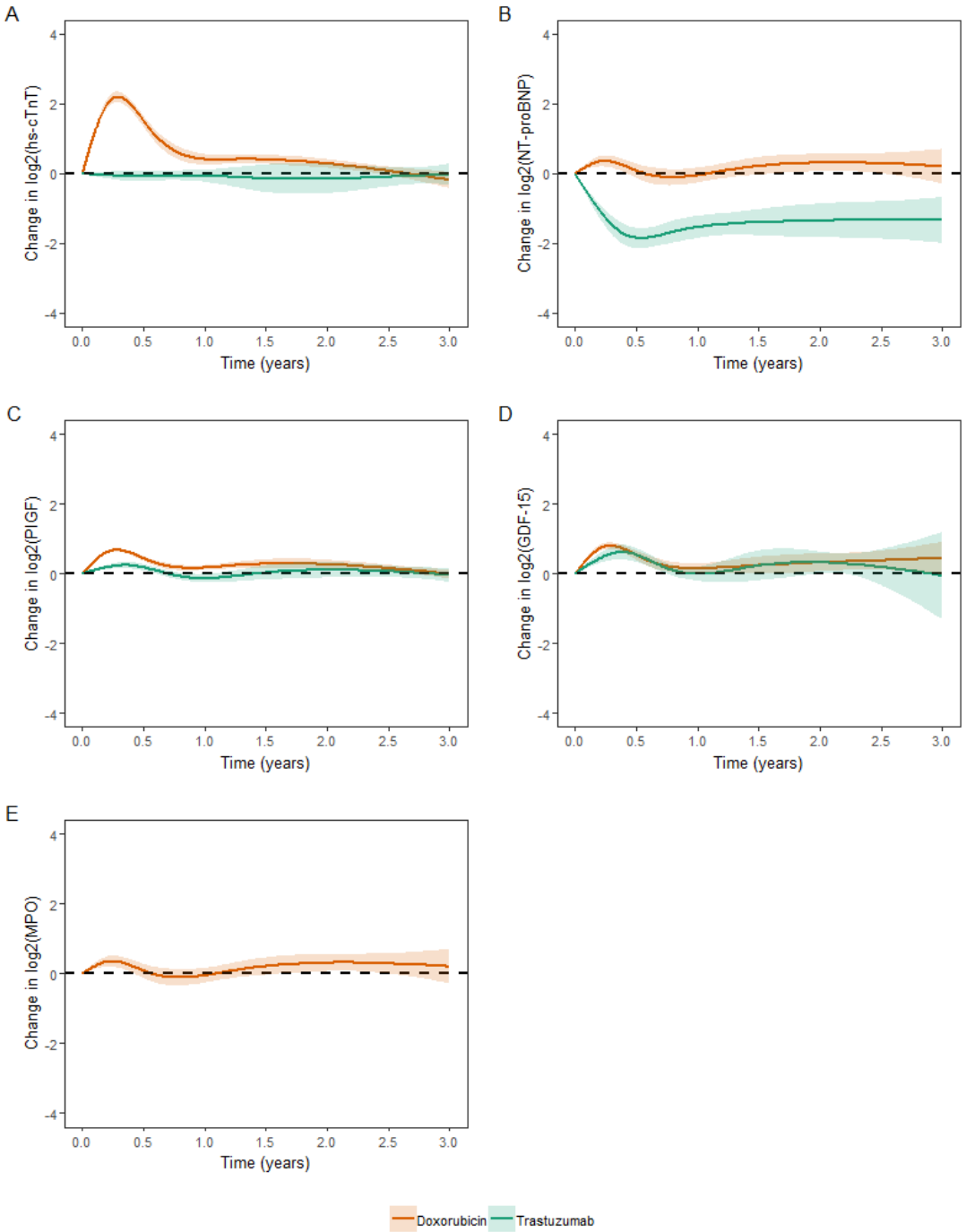

Figure S4. Mean estimated changes in biomarkers over time according to cancer therapy regimen; the Doxorubicin group includes both those treated with doxorubicin alone and sequential doxorubicin and trastuzumab therapy. The solid line represents mean estimated changes over time and the width of the surrounding band represents the corresponding 95% confidence interval; biomarker levels were log2 transformed (a unit increment from baseline should be interpreted as doubling); (A) hs-cTnT, (B) NT-proBNP, (C) PIGF, (D) GDF-15, (E) MPO; GDF-15=Growth differentiation factor 15; hs-cTnT= High sensitivity cardiac troponin T; MPO=Myeloperoxidase; NT-proBNP= N-terminal pro-B-type natriuretic peptide; PIGF=Placental growth factor

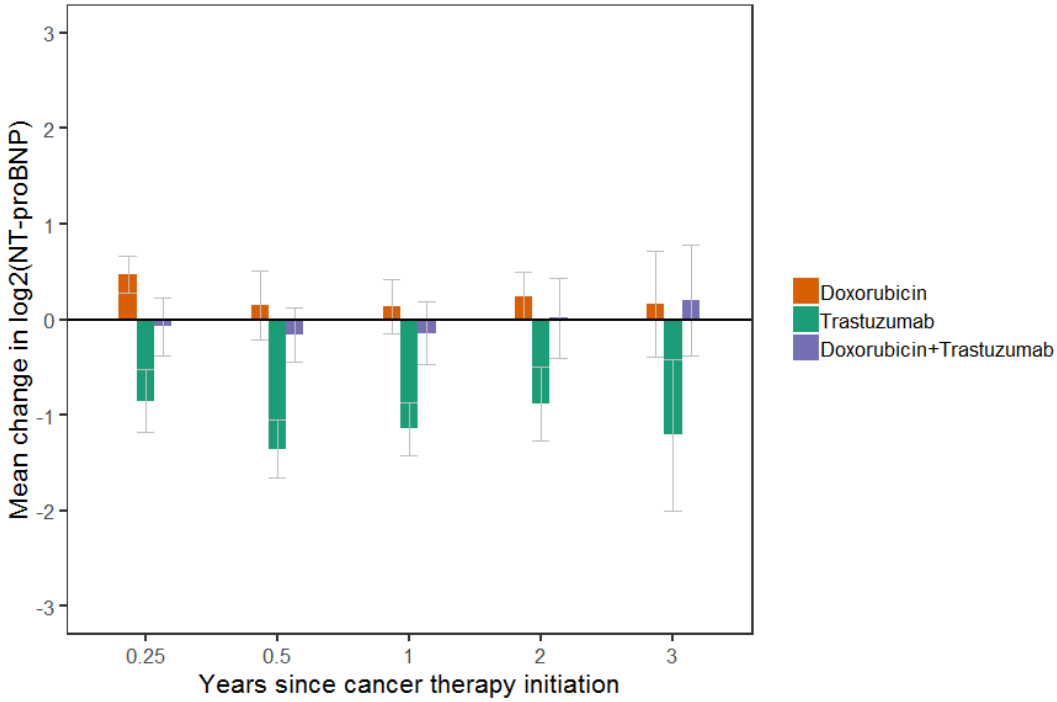

Figure S5. Covariate-adjusted marginal mean estimates of NT-proBNP change at 3, 6, 12, 24 and 36 months after initiation of cancer therapy according to cancer therapy regimen. Covariates include baseline NT-proBNP and baseline variables including age, hypertension, BMI, smoking; the effect of time was allowed to differ according to cancer therapy regimen by including a time*treatment interaction term