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Early Increases in Multiple Biomarkers Predict Subsequent Cardiotoxicity in Patients With Breast Cancer Treated With Doxorubicin, Taxanes, and Trastuzumab

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Objectives The aim of this study was to determine if individual or multiple biomarkers are associated with cardiotoxicity in patients with breast cancer undergoing cancer therapy.

Background Current methods to identify patients at risk for cardiotoxicity from cancer therapy are inadequate.

Methods We measured 8 biomarkers in a multicenter cohort of 78 patients with breast cancer undergoing doxorubicin and trastuzumab therapy: ultrasensitive troponin I (TnI), high-sensitivity C-reactive protein (CRP), N-terminal pro–B-type natriuretic peptide (NT-proBNP), growth differentiation factor (GDF)-15, myeloperoxidase (MPO), placental growth factor (PlGF), soluble fms-like tyrosine kinase receptor (sFlt)-1, and galectin (gal)-3. Cardiotoxicity, defined by the Cardiac Review and Evaluation Committee criteria, was assessed every 3 months for up to 15 months. Hazard ratios (HRs) of cardiotoxicity risk were assessed for each biomarker at baseline, at visit 2 (3 months), and as a function of the difference between visit 2 and baseline. Joint models were assessed for the most promising biomarkers.

Results TnI, CRP, GDF-15, MPO, PlGF, and sFlt-1 levels increased from baseline to visit 2 ($p < 0.05$). A greater risk of cardiotoxicity was associated with interval changes in TnI (HR: 1.38 per SD; 95% confidence interval: 1.05 to 1.81; $p = 0.02$) and MPO (HR: 1.34 per SD; 95% confidence interval: 1.00 to 1.80; $p = 0.048$) and in models combining both markers ($p = 0.007$ and $p = 0.03$, respectively). The risk of cardiotoxicity was 46.5% in patients with the largest changes in both markers (ΔTnI >121.8 ng/l; ΔMPO >422.6 pmol/l).

Conclusions Early increases in TnI and MPO levels offer additive information about the risk of cardiotoxicity in patients undergoing doxorubicin and trastuzumab therapy. Independent validation of these findings is necessary before application to clinical practice. (J Am Coll Cardiol 2014;63:809–16) © 2014 by the American College of Cardiology Foundation

Highly-effective cancer drugs such as doxorubicin and trastuzumab (Herceptin Genentech, San Francisco, California) are used widely in the treatment of patients with HER2-positive breast cancer and have led to important gains in survival. However, these agents carry a significant risk of cardiovascular morbidity. Clinical trial data suggest that, when used in combination, treatment with doxorubicin and trastuzumab

See page 817

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Abbreviations and Acronyms

CI = confidence interval
CRP = C-reactive protein
gal = galectin
GDF = growth differentiation factor
HF = heart failure
HR = hazard ratio
LVEF = left ventricular ejection fraction
MPO = myeloperoxidase
NT-proBNP = N-terminal pro–B-type natriuretic peptide
PlGF = placental growth factor
sFlt = soluble fms-like tyrosine kinase receptor
TnI = troponin I

results in an incidence of cardiac dysfunction on the order of 18%, with 2% to 4% developing severe symptomatic heart failure (HF) (1–3). Retrospective analyses suggest a greater risk of dysfunction in the nonclinical trial population. Data from the Cancer Research Network showed that the use of anthracyclines and trastuzumab in combination was associated with a >7-fold increased risk of HF or cardiomyopathy (4).

As such, there remains a fundamental need to identify patients with cancer undergoing treatment with these agents who are at high risk for cardiac complications. Early identification of subclinical cardiac dysfunction could enable the institution of cardioprotective strategies, prevent the interruption or discontinuation of necessary cancer therapy, and reduce early and late cardiovascular and oncological morbidity and mortality.

The methods currently used to identify patients at risk for cardiotoxicity are inadequate. Screening of patients before treatment and monitoring of cardiac function during therapy have relied traditionally on left ventricular ejection fraction (LVEF) (5). However, assessment of LVEF lacks the sensitivity to detect early subclinical changes or predict subsequent declines in function with treatment (6,7). Newer metrics are needed to identify vulnerable patients during the pre-clinical stage of cardiotoxicity; in other cardiovascular diseases, the assessment of multiple biomarkers has been shown to be of incremental utility in identifying patients at increased risk for adverse outcomes (8–10).

The overall objective of this study was to determine the potential utility of biomarkers for the early identification of patients with breast cancer at risk for cardiac dysfunction. We evaluated the associations between 8 biomarkers and the risk of subsequent cardiotoxicity in a multicenter cohort of 78 patients with breast cancer undergoing therapy with doxorubicin and trastuzumab. We hypothesized that the following cardiovascular biomarkers could be mechanistically relevant to cardiotoxicity with cancer therapy: ultrasensitive troponin I (TnI) (cardiomyocyte injury), high-sensitivity C-reactive protein (CRP) (inflammation), N-terminal pro–B-type natriuretic peptide (NT-proBNP) (neurohormonal activation), growth differentiation factor (GDF)-15 (inflammation and oxidative stress), myeloperoxidase (MPO) (oxidative stress), placental growth factor (PlGF) (angiogenesis), soluble fms-like tyrosine kinase receptor (sFlt)-1 (vascular remodeling), and galectin (gal)-3 (fibrosis). Our objectives were to determine whether individual biomarker levels, early changes in biomarker levels, or a combination of biomarkers could predict subsequent cardiotoxicity in patients treated with doxorubicin and trastuzumab.

Methods

Study population. The study population consisted of outpatients with HER2-positive breast cancer recruited across 4 centers as previously reported (11,12). Patients with HER2-positive breast cancer who were scheduled to undergo adjuvant therapy with an anthracycline-containing regimen followed by taxanes and trastuzumab were included. This regimen typically consisted of doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) every 3 weeks for 4 cycles. At 3 months, paclitaxel (80 mg/m^2) was administered concurrently with trastuzumab every week for 12 weeks; trastuzumab was then continued every 3 weeks to complete 1 year of therapy. Patients with an LVEF <50% before chemotherapy or those who were unwilling or unable to provide informed consent were excluded. This study consisted of participants with biomarker data who completed the protocol.

Participants were studied before chemotherapy and at standardized intervals every 3 months during anthracycline, paclitaxel, and trastuzumab therapy using serial questionnaires and echocardiograms for a total of 6 study visits per participant (Fig. 1). Transthoracic echocardiograms were acquired using Vivid 7 or E9 (GE Healthcare, Milwaukee, Wisconsin) at these time intervals. Blood samples were obtained at baseline, 3 months, and 6 months, processed, and stored at −80°C until the time of assay. The primary endpoint of cardiotoxicity was the Cardiac Review and Evaluation Committee definition of trastuzumab-associated cardiotoxicity, that is, a reduction in LVEF of ≥5% to <55% with symptoms of HF or an asymptomatic reduction of LVEF of ≥10% to <55% (1).

All participants provided written informed consent, and the protocol was approved by the institutional review boards of the participating institutions.

Echocardiography. Digital echocardiograms were analyzed on the EchoPAC workstation (GE Healthcare). Left ventricular end-diastolic and -systolic volumes were obtained using

School, Boston, Massachusetts; and the §§Gillette Center for Breast Cancer, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts.
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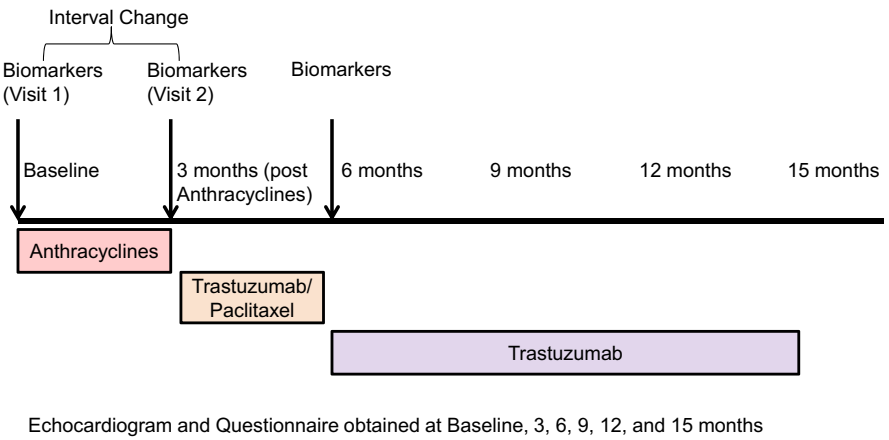

Figure 1 **Study Protocol**

Participants were studied before chemotherapy and at standardized intervals every 3 months during anthracycline, paclitaxel, and trastuzumab therapy using serial questionnaires and echocardiograms for a total of 6 study visits per participant. Biomarkers were measured at baseline (visit 1), 3 months (visit 2), and 6 months.

Simpson's method of discs in the apical 4- and 2-chamber views as recommended by the American Society of Echocardiography (13). Ejection fraction was calculated from left ventricular volumes as follows: (end-diastolic volume − end-systolic volume)/end-diastolic volume × 100%. All tracings were made by a single observer at a centralized reading center who was blinded to all other clinical or biomarker data.

Laboratory analyses. Biomarkers were measured from banked plasma stored at −80°C until assay. Ultrasensitive TnI and NT-proBNP were measured on the Dimension Vista 500 Intelligent Laboratory System (Siemens Healthcare Diagnostics, Deerfield, Illinois) using a 1-step, homogeneous, sandwich chemiluminescent pre-commercial immunoassay on the basis of Loci technology. High-sensitivity CRP was measured using a standard Architect immunoassay (Abbott Laboratories, Abbott Park, Illinois). GDF-15, MPO, PlGF, sFlt-1, and gal-3 were all measured using prototype Architect chemiluminescent 2-step microparticle-based immunoassays (Abbott Laboratories). Full details are provided in the Online Appendix.

Statistical methods. Data are described using summary statistics, that is, medians with interquartile range and means with SDs for continuous variables and proportions for categorical variables. The Wilcoxon test was used to assess the change in each biomarker concentration from baseline to 3 months (i.e., visit 2, the first follow-up visit). The majority of biomarkers were remeasured at the 3-month visit (in 90% to 95% of patients) and in 5% to 10% of patients at the 6-month visit. This follow-up measurement is thus described as the visit 2 measurement, with the understanding that it primarily represents a change from baseline to 3 months. Sensitivity analyses revealed that our results were very similar, irrespective of whether we defined visit 2 based strictly on only the 3-month visit or included those patients who had a 6-month value despite a missing 3-month value.

Given that the objective of this study was to focus specifically on early changes in markers, we determined the association between the risk of cardiotoxicity and biomarker levels at baseline and visit 2 and changes in levels from baseline to visit 2. To better compare biomarkers, the predictors were normalized by the SD in either the biomarker level or the change in level as appropriate. Candidate biomarkers with p values <0.20 in univariable models were then individually added to a model containing either the baseline value or change in TnI to determine whether there was additional information that could be obtained from markers in combination. Kaplan-Meier plots were used to illustrate the incidence of cardiotoxicity according to interval changes in biomarkers. To conceptualize the combined effect of the marker changes, we used the final model to predict cardiotoxicity over time at the 10th, 50th, and 90th percentile of the interval change in the 2 biomarkers in the final model. Analyses were performed using interval-censored time-to-event methods and a parametric Weibull survival model because cardiotoxicity was evaluated at visits separated by 3-month intervals and was thus both left and right censored (14).

For a 2-sided type I error rate of 0.05, a sample size of 78 patients, and an event rate of approximately 30%, this study would have 80% power to detect relatively large hazard ratios (HRs), on the order of 1.78, per SD in biomarker or biomarker change (PASS 2008, Kaysville, Utah). Analyses were performed using R 2.15 (R Development Core Team, Vienna, Austria) and the R package Icens (Gentleman and Vandal, Bioconductor, and R Project for Statistical Computing).

Results

Patient population. The baseline characteristics of the study cohort are shown in Table 1. Over a maximal follow-up

Table 1 Patient Characteristics (N = 78)

Age (yrs)	50.0 (42.0–56.8)
Body mass index (kg/m^2)	25.1 (22.2–27.2)
Tobacco use	7 (9)
Breast cancer side	
Left	38 (49)
Bilateral	6 (8)
Hypertension	21 (27)
Diabetes	1 (1)
Hyperlipidemia	18 (23)
Radiotherapy	46 (60)
Use of an ACE-inhibitor	13 (17)
Use of a beta-blocker	10 (13)
Baseline LVEF (IQR)	64 (61–68)

Values are median (interquartile range) or n (%).
ACE = angiotensin-converting enzyme; LVEF = left ventricular ejection fraction.

period of 15 months, there were a total of 23 outcomes (24%) as defined by the Cardiac Review and Evaluation Committee criteria for cardiotoxicity. The median change in LVEF among those patients who experienced cardiotoxicity was 15% (interquartile range: 12% to 17%). One of these patients had a decline in ejection fraction of ≥5% to <55% with symptoms. Of the remaining 22 patients, 18 had a decline of ≥10% to <55% without symptoms and 4 patients had a decline of ≥10% to <55% with symptoms. The maximal changes in ejection fraction according to cardiotoxicity are displayed graphically in Online Figure 1. Among the participants who experienced cardiotoxicity, the median time to event was 7.9 months (interquartile range: 5.5 to 11.4 months).

Summaries of baseline levels of and interval changes in biomarkers are provided in Table 2. Levels of TnI, CRP, GDF-15, MPO, PlGF, and sFlt-1 increased significantly from baseline to visit 2 (all $p < 0.05$).

Association between individual biomarkers and incident cardiotoxicity. We then examined the association between baseline levels of biomarkers and risk of subsequent cardiotoxicity (Table 3). The risk of cardiotoxicity was not significantly associated with baseline levels of any biomarker, although MPO was of marginal significance ($p = 0.052$). When examining biomarker concentrations at visit 2 (Table 3), only TnI was statistically associated with risk of cardiotoxicity (HR: 1.36; 95% confidence interval [CI]: 1.07 to 1.73; $p = 0.012$; per each increase in SD), as has been previously established (12).

In comparison, interval changes in several biomarkers from baseline to visit 2 (3 months) were associated with subsequent cardiotoxicity (Table 3). For each increase in SD in TnI (a change of 106.8 ng/l), there was a nearly 40% increased risk of subsequent cardiotoxicity (HR: 1.38; 95% CI: 1.05 to 1.81; $p = 0.020$). MPO had a similar effect size (HR: 1.34; 95% CI: 1.00 to 1.80; $p = 0.048$; per each increase in SD [a change of 355.6 pmol/l]). The distribution of interval changes in these biomarkers and the incidence of cardiotoxicity are shown as Kaplan-Meier plots (Online Figs. 2 and 3). On the basis of these models, the predicted probabilities of cardiotoxicity as a function of time for the 10th, 50th, and 90th percentiles of change in TnI and MPO are displayed in Figures 2A and 2B. At each time point, those patients at the 90th percentile of change in TnI or MPO had the greatest predicted risk of cardiotoxicity, with little difference in the predicted risk between the 10th and 50th percentile groups. For patients at the 10th (−0.9 ng/l) or 50th (13.9 ng/l) percentiles of ΔTnI or the 10th (−64.8 pmol/l) or 50th (26.3 pmol/l) percentiles of ΔMPO, the probability of cardiotoxicity by 15 months was 23.6% to 26.5%, respectively. For patients at the 90th (121.8 ng/l or 422.6 pmol/l) percentile for ΔTnI or ΔMPO, the probability of cardiotoxicity by 15 months was 34.2% to 36.1%.

We noted that increases in GDF-15 and gal-3 levels were associated with similar effect sizes as TnI and MPO, with a HR of 1.33 for each biomarker, although neither was statistically significant ($p = 0.118$ and $p = 0.195$ per each increase in SD). There was no evidence of an association between an early interval change (baseline to visit 2) in NT-proBNP, CRP, PlGF, or sFlt-1 levels and subsequent cardiotoxicity.

Table 2 Baseline and Interval Changes in Biomarker Levels Between Baseline and Visit 2

Biomarker	Mean Baseline Levels	Median Baseline Levels	Difference*	p Value†
TnI (ng/l)	6.2 ± 13.7	1.3 (0.7 to 4.0)	13.9 (2.6 to 31.6)	<0.001
NT-proBNP (pg/ml)	104.0 ± 114.2	71.0 (37.0 to 133.3)	−3.0 (−38.0 to 26.8)	0.61
CRP (mg/l)	3.5 ± 5.0	1.7 (0.63 to 3.92)	0.6 (−0.2 to 4.3)	<0.001
GDF-15 (ng/l)	575.4 ± 291.5	500.4 (348.9 to 732.7)	222.0 (27.7 to 467.5)	<0.001
MPO (pmol/l)	156.3 ± 159.1	107.1 (76.7 to 173.4)	26.3 (−27.3 to 114)	0.008
PlGF (pg/ml)	20.8 ± 5.4	19.6 (17.5 to 23.3)	5.3 (2.9 to 9.3)	<0.001
sFlt-1 (pg/ml)	343.4 ± 364.6	236.4 (215.4 to 272.2)	33.3 (−14.7 to 87.3)	<0.001
gal-3 (ng/ml)	18.1 ± 18.3	13.8 (10.8 to 18.2)	0.5 (−1.5 to 2.2)	0.14

Values are mean ± SD or median (interquartile range). *Median difference represents the difference between visit 2 and baseline levels. †p value according to Wilcoxon rank sum test.
CRP = C-reactive protein; gal = galectin; GDF = growth differentiation factor; MPO = myeloperoxidase; NT-proBNP = N-terminal pro–B-type natriuretic peptide; PlGF = placental growth factor; sFlt = soluble fms-like tyrosine kinase receptor; TnI = troponin I; other abbreviations as in Table 1.

Table 3 Risk of Cardiotoxicity According to Biomarker Levels at Baseline, Visit 2, and Early Interval Changes (Baseline to Visit 2)

	Baseline		Visit 2		Interval Change	
Biomarker	HR (95% CI)*	p Value†	HR (95% CI)*	p Value†	HR (95% CI)*	p Value†
TnI	1.21 (0.92–1.61)	0.177	1.36 (1.07–1.73)	0.012	1.38 (1.05–1.81)	0.020
NT-proBNP	0.78 (0.48–1.25)	NS	0.89 (0.59–1.35)	NS	1.11 (0.80–1.54)	NS
CRP	1.18 (0.85–1.63)	NS	1.07 (0.72–1.60)	NS	0.95 (0.52–1.73)	NS
GDF-15	0.90 (0.59–1.37)	NS	1.26 (0.89–1.78)	0.189	1.33 (0.93–1.92)	0.118
MPO	0.66 (0.44–1.00)	0.052	1.23 (0.93–1.62)	0.149	1.34 (1.00–1.80)	0.048
PlGF	0.88 (0.55–1.40)	NS	1.17 (0.82–1.65)	NS	1.16 (0.77–1.73)	NS
sFlt-1	1.05 (0.70–1.56)	NS	0.76 (0.54–1.06)	0.109	0.75 (0.51–1.10)	0.139
Gal-3	0.70 (0.44–1.11)	0.128	0.94 (0.62–1.41)	NS	1.33 (0.86–2.05)	0.195

*HR expressed per SD increase in biomarker level. †$p > 0.20$ are shown as NS.
CI = confidence interval; HR = hazard ratio; NS = not significant; other abbreviations as in Table 2.

Association between multiple biomarkers and incident cardiotoxicity. Elevations in absolute levels of TnI at visit 2 in this cohort were previously reported to be associated with an elevated risk of cardiotoxicity (12). We thus considered whether the addition of other biomarkers could provide incremental information to what could be ascribed to either the TnI level at visit 2 or changes in TnI levels alone. For these analyses, we selected biomarkers with some evidence of association with cardiotoxicity in the individual change models (MPO, GDF-15, gal-3; all with $p < 0.20$ in the individual models).

Jointly, interval changes in MPO and TnI levels provided additive value in predicting subsequent cardiotoxicity, with HRs of 1.36 (95% CI: 1.04 to 1.79; $p = 0.03$) and 1.43 (95% CI: 1.10 to 1.86; $p = 0.007$), respectively, per each SD increase in interval change. To illustrate the effects of 2 biomarkers in combination, Figures 3A and 3B show the estimated probability of cardiotoxicity for patients stratified into 3 groups. The "none high" group lacked large elevations in either biomarker (i.e., quantiles of 0.10 and 0.50 for each biomarker). The "one high" group had elevated changes in 1 biomarker (i.e., quantile of 0.90 in either ΔTnI or ΔMPO and quantile of 0.10 or 0.50 for the other biomarker). The "two high" group had elevated changes in both biomarkers (quantile of 0.90 for both ΔTnI and ΔMPO). Because the "none high" and "one high" groups incorporated predictions from our multiple biomarker model for several different combinations of biomarker levels, the figures show the range of predictions as a solid block. As shown in Figures 3A and 3B, the probability of cardiotoxicity by 15 months in patients without elevations of either biomarker ranged from 20.4% to 23.4%. The probability of cardiotoxicity in patients with 1 biomarker with an elevated change ranged from 31.6% to 33.9% and for patients with elevated changes in both biomarkers was 46.5%. At all time points, patients with elevated changes in both biomarkers had the greatest risk of cardiotoxicity. These findings suggest that the combination of multiple markers, such as MPO and TnI,

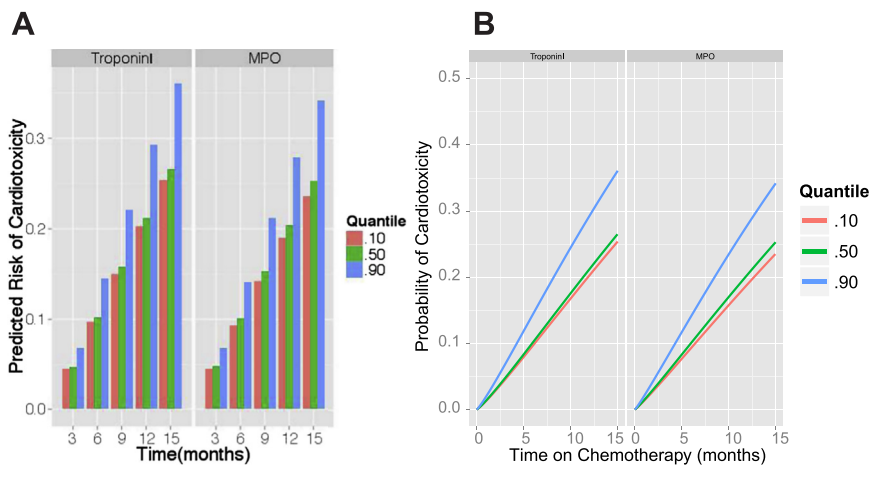

Figure 2 Model-Based Probability of Cardiotoxicity According to Percentile Changes in TnI and MPO Between Baseline and Visit 2

(A) Bars represent the probability of cardiotoxicity at the 10th, 50th, and 90th percentiles of interval changes (baseline and visit 2) in TnI and MPO. These quantiles were −0.9, 13.9, and 121.8 ng/l for TnI and −64.8, 26.3, and 422.6 pmol/l for MPO. **(B) Curves** represent the probability of cardiotoxicity at the 10th, 50th, and 90th percentiles of interval changes (baseline and visit 2) in TnI and MPO. MPO = myeloperoxidase; TnI = troponin I.

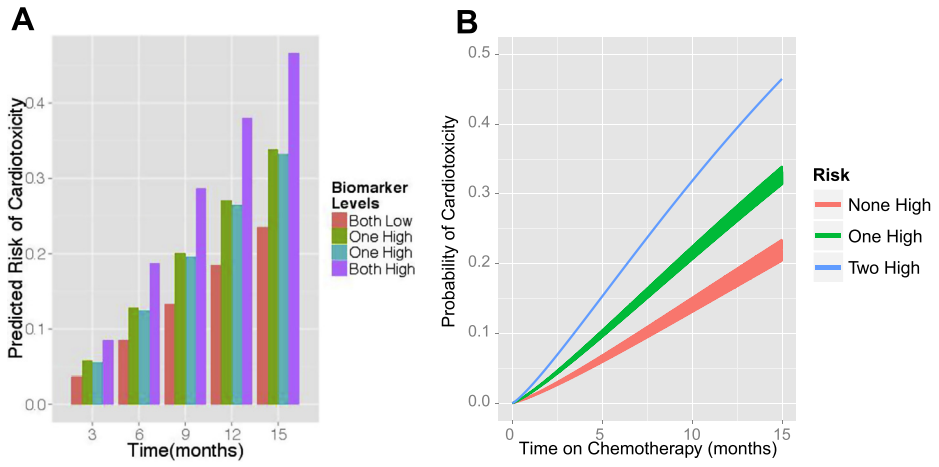

Figure 3 **Model-Based Probability of Cardiotoxicity According to Changes in MPO and TnI Levels**

(A) Bars represent the probability of cardiotoxicity according to changes in biomarkers in both TnI and MPO at standardized time intervals. "Both Low" refers to no elevations in either biomarker (quantiles of 0.1, 0.5), "One High" notes elevations in 1 biomarker (quantile of 0.9 in either TnI or MPO and quantile of 0.1 or 0.5 for the other biomarker), and "Both High" notes elevations in both biomarkers (quantiles of 0.9). **(B) Curves** represent the probability of cardiotoxicity according to changes in biomarkers, where "None," One High," and "Two High" represent the same categories as in **A**. The results are displayed as **shaded areas** on the graph representing the upper and lower values of the probability region for each of the 3 categories. Abbreviations as in Figure 2.

may improve the detection of doxorubicin and trastuzumab–induced cardiotoxicity.

The addition of other candidate biomarkers such as GDF-15 (HR: 1.19; 95% CI: 0.83 to 1.70; $p = 0.35$) or gal-3 (HR: 1.31; 95% CI: 0.82 to 2.1; $p = 0.26$) to a model containing ΔTnI did not yield significant findings. Because the TnI concentration at visit 2 was previously shown to be associated with cardiotoxicity, in sensitivity analyses, we also considered joint models containing early changes in MPO, GDF, or gal-3 levels with the TnI value at visit 2. Again, only the model containing ΔMPO (HR: 1.38; 95% CI: 1.05 to 1.81; $p = 0.023$) retained significance for the added biomarker; here, the HR for troponin was 1.42 (95% CI: 1.10 to 1.81; $p = 0.006$). The HRs for ΔMPO in the model with ΔTnI or with the absolute level of TnI at visit 2 were remarkably similar.

In our sensitivity analyses, using the criteria for cardiotoxicity as defined by a decline in LVEF of at least 10% to <50%, as in the HERA trial (2), there were 11 observed events. No biomarkers were independently associated with subsequent cardiotoxicity at a level of $p < 0.05$ using this criterion. However, using the criterion for cardiotoxicity as defined by a decline in LVEF of more than 10%, as in the BCIRG006 trial (3), 36 participants met this definition. Here, our associations between interval changes in MPO levels and subsequent cardiotoxicity remained significant (HR: 1.32; 95% CI: 1.1 to 1.6; $p = 0.011$). In additional sensitivity analyses, we considered a $\log_2$ transformation of a rescaled predictor (to accommodate negative values), which yielded similar results, albeit generally with somewhat larger p values.

Discussion

Cardiotoxicity associated with cancer therapy is a growing problem, magnified by the lack of ability to identify patients at increased risk for cardiotoxicity, both before and early in therapy. In this study, we ascertained that early changes in TnI and MPO levels are associated with subsequent cardiotoxicity; the combination of multiple markers, including TnI and MPO, may have joint utility in predicting subsequent cardiotoxicity; and baseline levels appear to have less prognostic significance. Overall, our results suggest that early interval changes in individual biomarkers may be of utility in predicting adverse cardiovascular outcomes with doxorubicin and trastuzumab.

Biomarkers in cardio-oncology have been shown to be of use in identifying patients with subclinical cardiotoxicity, particularly related to anthracycline use. One high-impact study in 703 patients with various malignancies undergoing high-dose anthracycline chemotherapy showed that multiple measures of plasma TnI obtained at various time points were predictive of subsequent cardiotoxicity (15), with a positive and negative predictive value of 84% and 99%, respectively.

This same group of investigators studied the utility of TnI in patients with breast cancer treated with trastuzumab (16); 78% of these subjects were previously exposed to anthracycline chemotherapy. Elevated TnI levels were independently associated with cardiotoxicity, with a HR of approximately 17. However, other investigators have noted that a large proportion of patients (67%) undergoing sequential therapy with doxorubicin, trastuzumab, and lapatinib have elevated TnI levels over the course of chemotherapy (17) and

determined that there was no significant association between maximal TnI level and change in LVEF.

Our findings suggest that there is an association between TnI positivity and subsequent dysfunction and provide evidence to support the importance of assessing changes in biomarkers over time. The utility of TnI appears to lie in evaluating levels after initial anthracycline chemotherapy exposure as opposed to baseline. Furthermore, changes in TnI levels with anthracycline exposure are associated with subsequent cardiotoxicity.

To our knowledge, we describe for the first time an association between early changes in MPO levels and subsequent cardiotoxicity induced by treatment with doxorubicin and trastuzumab. MPO is an enzyme secreted by polymorphonuclear leukocytes that is proatherogenic and pro-oxidant, causing lipid peroxidation, scavenging of nitric oxide, and inhibition of nitric oxide synthase (18,19). In patients with acute coronary syndromes, elevated levels of MPO are predictive of adverse outcomes, providing additive value to troponinT (18). Studies in HF also suggest that MPO is related to an increased risk of more severe disease (20). Because oxidative stress is hypothesized to be central to the mechanisms of doxorubicin cardiotoxicity (21), it is biologically plausible that elevated MPO levels after exposure to doxorubicin are associated with subsequent cardiac dysfunction (22). Our findings also suggest that MPO can be used in combination with TnI to identify a subgroup of patients that are at a substantially increased risk for cardiotoxicity. MPO and TnI appear to offer additive information, with no significant correlation ($R = -0.13$, $p = 0.27$), suggesting that these biomarkers are reflective of orthogonal biologic axes. With future study, these markers in combination may be helpful to further gauge cardiovascular risk.

In terms of other biomarkers, there were no significant associations between cardiotoxicity and elevated levels of gal-3 and GDF-15. Gal-3 is a 26-kDa protein that is expressed by macrophages and is believed to be a mediator of profibrotic pathways, stimulating cardiac fibroblasts to proliferate and deposit collagen. Gal-3 concentrations are elevated in patients with acute HF and are predictive of an increased risk of adverse outcomes (23). GDF-15 is a member of the transforming growth factor β cytokine superfamily that is produced in response to oxidative stress, inflammation, and injury (24) and is an emerging marker in acute coronary syndromes and HF, although its concentrations may be elevated in patients with malignancies. We found no significant association between either GDF-15 or gal-3 and cardiotoxicity, but further study in larger sample sizes is justifiable.

The lack of an association between CRP levels and cardiotoxicity is in agreement with the findings of others (19). Although early changes in NT-proBNP levels were not associated with cardiotoxicity, our results do not exclude the possibility that late changes are predictive of subsequent adverse cardiovascular events. Neither angiogenic factor sFlt-1 nor PlGF was associated with subsequent cardiotoxicity.

Study limitations. Although our study is one of the larger biomarker studies in patients treated with doxorubicin followed by trastuzumab, the sample size and the number of cardiotoxicity events means that we had power to detect only large associations (HR of approximately 1.8 per SD change on a linear scale). In fact, the HRs that were detected with statistical significance were of lower magnitude, on the order of 1.3 to 1.4. Furthermore, the SD in the interval change in TnI represented a >300% change, suggesting that this change could not be explained by biological variability alone (25). We also acknowledge that larger sample sizes may be needed to optimally define the metric describing the association between biomarker changes and cardiotoxicity (e.g., nonlinear associations). While TnI and MPO were the most promising candidates in the current study, additional biomarkers likely deserve further study in larger sample sizes. Given our small sample size, we were precluded from adequately assessing the impact of all potential confounders. It is also critical that our findings are further validated before application to clinical practice. Finally, it is not possible to differentiate whether our findings are secondary to anthracyclines, trastuzumab, or a combination of the 2 agents. To answer this question, future biomarker studies in patients treated with each agent individually are necessary. Nonetheless, these findings are very relevant to a population of patients with breast cancer undergoing combination therapy.

Conclusions

Our study confirms that TnI is associated with subsequent cardiac dysfunction and HF in patients with breast cancer undergoing sequential therapy with doxorubicin and trastuzumab. We found that MPO is a potential marker of incident cardiac dysfunction in this population. Finally, our results suggest that a multimarker approach may increase the sensitivity of cardiotoxicity risk prediction in patients treated with anthracyclines, taxanes, and trastuzumab.

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Key Words: cardio-oncology ■ chemotherapy cardiotoxicity ■ trastuzumab cardiotoxicity.

APPENDIX

For the supplemental appendix and figures, please see the online version of this article.