Predictive Biomarkers and Personalized Medicine

Troponin I and C-Reactive Protein Are Commonly Detected in Patients with Breast Cancer Treated with Dose-Dense Chemotherapy Incorporating Trastuzumab and Lapatinib

Patrick G. Morris1, Carol Chen1, Richard Steingart1, Martin Fleisher1, Nancy Lin2, Beverly Moy3, Steven Come4, Steven Sugarman1, Alyson Abbruzzi1, Robert Lehman1, Sujata Patil1, Maura Dickler1, Heather L. McArthur1, Eric Winer2, Larry Norton1, Clifford A. Hudis1, and Chau T. Dang1

Abstract

Purpose: There are no validated methods of early detection of cardiotoxicity from trastuzumab (T) following anthracycline-based chemotherapy. Currently changes in left ventricular ejection fraction (LVEF) are assessed but this approach has limited sensitivity and specificity. Within a prospective feasibility study of dose-dense (dd) doxorubicin and cyclophosphamide (AC) — weekly paclitaxel (P) with T and lapatinib (L), we included a preplanned analysis of correlative cardiac Troponin I (cTnI) and C-reactive protein (CRP) as early biomarkers of cardiotoxicity.

Experimental Design: As previously described, patients received ddACx 4—PTL—TL. LVEF was assessed at months 0, 2, 6, 9, and 18 and cTnI and CRP measured every 2 weeks during chemotherapy then at months 6, 9, and 18. These biomarkers were correlated with changes in LVEF.

Results: Ninety-five patients enrolled. Overall, 3 (3%) patients withdrew during AC and 41 (43%) withdrew during PTL—TL, mostly due to diarrhea. Median LVEF was 68% (baseline), 69% (month 2), 65% (month 6), 65% (month 9), and 65% (month 18). The majority (67%) had a detectable cTnI during the study. The proportion of detectable cTnIs increased over time; 4% at baseline, 11% at month 2, and 50% at month 3. The timing of these detectable cTnIs preceded maximum-recorded decline in LVEF. However, overall, maximum cTnI levels did not correlate with LVEF declines. A detectable CRP was seen in 74/95 (78%) but did not correlate with LVEF declines.

Conclusion: In patients receiving ddAC—PTL, cTnIs are commonly detected. These elevations may precede changes in LVEF but, as assessed in this trial, do not predict CHF.

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Introduction

The incorporation of trastuzumab (T) into anthracycline-based chemotherapy has improved survival in early breast cancer for patients with overexpression or amplification of the human epidermal growth factor receptor-2 (HER2; ref. 1). However, this approach is associated with a relatively infrequent but clinically significant risk of long-term cardiotoxicity (1). The potential for congestive heart failure (CHF) with T was first appreciated in the metastatic setting (1). As a surrogate for clinical CHF, the large phase III studies of adjuvant T incorporated rigorous cardiac monitoring to detect asymptomatic declines in left ventricular ejection fraction (LVEF). It is now clear that these asymptomatic declines occur relatively frequently. For example, in the combined analysis of NSABP B-31 and N9831, there was a patient drop-out rate of 6.7% mainly due to numerically “significant” (based on preplanned rules), but clinically asymptomatic, LVEF declines after conventionally scheduled doxorubicin and cyclophosphamide (AC; ref. 2). In addition, of those eligible for T, 31.4% discontinued therapy before completion of the planned year of treatment predominantly because of similar declines in LVEF (2).

In contrast to these findings, we previously demonstrated that AC delivered once every 2 weeks (dd) is associated with a low rate of asymptomatic decline in LVEF (3). In addition, in an exploratory analysis of CALGB 9741, which demonstrated the survival advantage of this approach, ddAC had one-half of the rate of long-term grade 3–4 cardiac events compared to conventionally scheduled AC (4, 5). Subsequently, we demonstrated that ddAC followed by paclitaxel (P) with
T was associated with an overall CHF rate of 1.4% (1/70 patients; ref. 6). Taken together, these findings suggest that dd anthracycline-based chemotherapy incorporating T is associated with a low risk of cardiotoxicity. However, to date all these studies have relied on LVEF changes to predict for acute CHF, which has limited sensitivity and specificity. Therefore, effective cardiac biomarkers are needed.

In a clinical trial, testing the feasibility of adding lapatinib to dose-dense (dd) AC followed by paclitaxel/T, we prospectively examined cardiac Troponin I (cTnI) as a biomarker of cardiotoxicity. Elevations in cTnI occurred commonly and appeared to be temporally related to anti-HER2 therapy. Abnormalities in cTnI of this magnitude have previously been shown to predict for LVEF dysfunction but did not in the short-term here. Given the routine use of T, and possible underappreciation of these cTnI elevations, our results have potentially important implications for clinical practice. Furthermore, our results could aid in the development of predictive models of cardiotoxicity, incorporating cTnI and other putative biomarkers.

Materials and Methods

As previously described, eligible patients had early breast cancer which overexpressed HER-2/neu regardless of nodal status or tumor size (12). Patients with serious medical illnesses including unstable angina, myocardial infarction, and CHF were excluded. Treatment consisted of ddAC—PTL—TL, with cTnI and CRP measurement every 2 weeks during chemotherapy and then at months 6, 9, and 18, and LVEF assessment by MUGA at similar timepoints (Fig. 1). Although not specified by the protocol, it is standard practice at our institutions for research blood tests to be drawn immediately before an administered treatment. Per protocol, patients were required to continue blood draws even if they had stopped treatment because of toxicity. Investigators were blinded to biomarker results until all patients had completed 18-month follow-up. After completion of chemotherapy (and during TL), patients treated with trastuzumab (T) had MUGA scans at 0, 2, 6, 9, and 18 months.

\[\text{cTnI} \text{ is a highly specific marker of myocardial damage. It is released by cardiac myocytes in proportion to the degree of cardiac injury, although rare elevations can occur with seizures, cirrhosis, renal failure, sepsis, and following rigorous exercise (7–9). Nonetheless, cTnI is an attractive biomarker as it is not detectable in the serum of healthy individuals and has been shown to quantitatively predict for mortality in patients with acute coronary syndrome (10).}\

C-reactive protein (CRP) is a sensitive inflammatory marker, which may represent an independent risk factor for cardiovascular disease (11). Therefore, within a prospective study at Memorial Sloan-Kettering Cancer Center (MSKCC) and Dana Farber/Harvard Cancer Center (DF/HCC) testing the feasibility of ddAC—weekly PTL and lapatinib (L), we included a preplanned analysis of both cTnI and CRP as early biomarkers of cardiotoxicity.

Translational Relevance

Although trastuzumab (T) has improved survival from early breast cancer that overexpresses the human epidermal growth factor receptor-2 (HER2), this agent is associated with the development of congestive heart failure (CHF). As a surrogate for CHF, left ventricular ejection fraction (LVEF) monitoring has been adopted into clinical practice. However, this approach has limited sensitivity and specificity as a predictor of the development of CHF. Therefore, effective cardiac biomarkers are needed.

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received radiation and endocrine therapy per standard guidelines.

Blood samples were analyzed in a centralized laboratory at the time of collection in each institution. The cTnI was measured using a fluorometric enzyme immunoassay analyzer (Tosoh Bioscience, Inc.) with a low-end sensitivity of 0.06 ng/mL (MSKCC) and 0.04 ng/mL (DF/HCC). Per institutional policy, a cTnI reading of ≥0.31 ng/mL was considered elevated in the setting of acute coronary syndrome, but any detectable cTnI (>0.06 ng/mL (MSKCC) and ≥0.04 ng/mL (DF/HCC)) was considered abnormal for the purposes of the study. The CRP was measured by particle-enhanced immunoturbidimetry, with a lower limit of detection of 0.02 mg/L (Beckman-Colter Diagnostics). Per institutional policy elevated CRP was defined as ≥0.8 mg/dL (MSKCC) and ≥0.3 mg/dL (DF/HCC). Changes in cTnI and CRP values were compared with the change in LVEF for all 95 enrolled patients over the study period using ANOVA or regression.

**Results**

From March of 2007 to April of 2008, we enrolled 95 patients as previously reported with baseline characteristics shown in Table 1 (3, 12). Overall, 3 of 95 (3%) patients withdrew during AC (1 expired after AC#2 due to viral

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics of patients (N = 95)</th>
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<tr>
<td><strong>All patients (N = 95)</strong></td>
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<tr>
<td>Age; median (years)</td>
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<tr>
<td>4–9</td>
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<td>Left-sided radiation</td>
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<tr>
<td>Right-sided radiation</td>
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<tr>
<td>Bilateral radiation</td>
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</tbody>
</table>

aIncludes 1 patient with prior mastectomy and subsequent ipsilateral recurrence.
Figures rounded to the nearest percentage.
pneumonia, 1 withdrew after AC#4 due to grade 3 fatigue, and 1 dropped out after AC#4 for personal reasons). In addition, 41 patients (43%) withdrew for toxicities related to PTL, mostly due to diarrhea (12). The LVEF results are shown in Figure 2.

Cardiac Troponin I

At baseline, 77 (81%) of patients had cTnI measured per protocol, of which 3 (4%) were detectable (Table 2). On completion of ddAC (and before PTL week 1) 87 (92%) of patients had cTnI measured, of which 10 (11%) were detectable. During PTL, 67% to 92% of blood draws occurred per protocol and detectable cTnIs were common (Figs. 3A and 4). The timing of these detectable cTnIs appeared to precede maximum decline in LVEF as shown in Figure 4A and B, which are time series graphs, showing mean LVEF and cTnI with standard errors at protocol-defined timepoints. For example, before week 5 of PTL, 41 patients (50%) had detectable cTnIs (Table 2). At month 6 (during TL), 60 (63%) patients completed cTnI blood draw, and none had a detectable cTnI. At month 9, 62 (65%) patients completed cTnI blood draw and only 1 patient (2%) had a detectable cTnI (0.06ng/ml at DF/HCC). At month 18, 22 (23%) patients had cTnI measured, none of whom had a detectable level. More than half, 64 (67%) of patients had a detectable cTnI at some point during the study. Nineteen patients had a single detectable cTnI and 45 (47%) had 2 or more detectable cTnIs; 16 had detectable cTnIs at 2 timepoints, and 19 had detectable cTnIs at 3 timepoints. Only 10 patients had 4 or more detectable cTnIs (5 had 4 detectable cTnIs, 3 had 5 detectable cTnIs, 1 had 6 detectable cTnIs, and 1 had 7 detectable cTnIs). None of these 10 patients developed CHF or was removed from treatment for asymptomatic decline in LVEF.

In the entire cohort, only 1 patient had an elevated cTnI by traditional acute coronary syndrome criteria (0.59 ng/mL) before AC#4. For her, a MUGA 1 week later was unchanged (LVEF 75%), but she died from liver failure from hepatitis B re-activation during subsequent treatment without evidence of CHF. Although detectable cTnIs were common, a subgroup of 31 (33%) patients had undetectable cTnIs throughout the study period. This group includes 17 patients who completed month 9 blood draw and 6 patients who completed month 18 blood draw.

C-reactive protein

At baseline, 74 (78%) of patients had CRP measured per protocol, of which 35 (47%) were elevated (Table 2). Elevations in CRP were common throughout treatment (Fig. 3B), although 21 patients had no elevation in CRP throughout the study period. Therefore, a subgroup of 9 (9%) patients was defined with undetectable cTnI and no elevation in CRP throughout the study period. On completion of ddAC (before PTL#1), 89 (94%) of patients had CRP measured and 42 (47%) were elevated. Following 4 weeks of PTL, 83 (87%) of patients had CRP measured and 46 (55%) were elevated. At month 6 (during TL), 58 (61%) patients completed CRP blood draw and 27 (47%) had elevated CRPs. At month 9, 25 (40%) and at month 18, 17 (61%) patients had elevated CRPs, respectively.

Congestive heart failure

Overall, at a median follow-up of 22 months, 3 of 95 (3%; 95% CI 0.7–9.0%) have developed clinical CHF. One patient, with no known cardiac risk factors, who received T but not L, had CHF at month 3 (baseline LVEF 68%, 68% post-ACx4, and 48% at month 3). She had an undetectable cTnI at baseline and a single detectable cTnI (of 0.08 ng/mL at DF/HCC) before AC#2. Repeat cTnI before AC#3, AC#4, PTL weeks #1 and #3 were undetectable. Her CRP levels were elevated at baseline (3.7 mg/dL, DF/HCC) and peaked before AC#2 (45.1 mg/dL) with subsequent values of 0.8, 0.9, 2.5, and 28.4 mg/dL. However, she had no blood draw immediately before the cardiac event.

A second patient, with no known cardiac risk factors, who received both T and L developed CHF at month 6 (baseline LVEF 52%, 56% post-ACx4, 24% at month 6). She had undetectable cTnI at baseline and throughout her treatment, including at month 6, although missed a single blood draw before week 3 of PTL. She had multiple elevations in CRP, including baseline (1.43 mg/dL, MSKCC)
and month 6 (1.1 mg/dL), to a maximum of 3.08 mg/dL before AC#2.

A third patient, who was a cigarette smoker with hypertension, received both T and L and developed CHF at month 12 (baseline LVEF >70%, >70% post-ACx4, 62% at month 6, 58% at month 10, and 25% at month 12). Her cTnI was undetectable throughout treatment until immediately before PTL week 7, when she stopped study treatment due to unacceptable diarrhea and no further cardiac biomarkers were collected. Her CRP was not elevated at baseline, peaked at 0.97 mg/dL (MSKCC) before AC#4, but subsequently normalized. All three patients have improved symptomatically with appropriate medical management, and there have been no cardiac deaths.

Significant asymptomatic LVEF decline

A decline in LVEF of >10% to <50% occurred in 9 (9%) patients. Before initiation of anti-HER2 therapy, one patient had a single LVEF decline following ACx4 (LVEF 68% at baseline, 47% post-ACx4, 64% at month 3, 67% at month 6, 67% at month 9), but no detectable cTnI, although she had a single detectable cTnI during PTL (0.06 ng/mL, MSKCC). After initiation of anti-HER2 therapy, 8 (8%) patients had LVEF decline of >10% to <50%. In 5 of these 8 patients, cTnI was not measured at the time of LVEF decline (months 6, 6, 7, 12, and 18, respectively); however, 2 of these 5 patients had cTnI elevations during PTL, which subsequently normalized. Three of 8 patients with significant LVEF decline had cTnI measured and none had detectable cTnI at the time of the event. One patient, who had 11 blood draws in total, had no detectable cTnI prior or following LVEF decline at month 4 (baseline LVEF 59%, 56% post-ACx4, 35% at month 4, 45% at month 5, 50% at month 6). The second patient with LVEF decline at month 6 had 2 detectable cTnIs (0.15 and 0.12 ng/mL, DF/HCC) during PTL, which normalized before the cardiac event (baseline LVEF 66%, 66% post-ACx4, 46% at month 6, 52% at month 7, 52% at month 9). The third patient with LVEF decline at month 9 had 4 detectable cTnIs during PTL (0.05, 0.16, 0.13, 0.06, and 0.05 ng/mL, DF/HCC), which normalized before the cardiac event (baseline LVEF 57%, 53% post-ACx4, 53% at month 6, 45% at month 9).

Predictive model for CHF and LVEF change

As shown in Figure 4, the timing of detectable cTnIs appeared to precede maximum decline in LVEF. We, therefore, investigated whether maximal detectable cTnI correlated overall with LVEF declines. The maximal percentage LVEF change was calculated using the following formula (maximum LVEF—minimum LVEF)/minimum LVEF and correlated with maximal detectable cTnI as a continuous variable. Overall, in the entire cohort, the maximal detectable cTnI (P = 0.11 MSKCC, P = 0.95 DF/HCC) was not associated with the maximal percentage LVEF change. We performed a similar analysis correlating the maximum LVEF decline from baseline (defined as baseline LVEF—minimum LVEF) with the maximal detectable cTnI as a continuous variable. Similarly, the maximal detectable

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### Table 2. Cardiac troponin I and CRP measured per protocol

<table>
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<tr>
<th>Timepoint</th>
<th>AC#1</th>
<th>AC#2</th>
<th>AC#3</th>
<th>AC#4</th>
<th>PTL Wk7</th>
<th>PTL Wk9</th>
<th>Mth 5</th>
<th>Mth 6</th>
<th>Mth 9</th>
<th>Mth 12</th>
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</thead>
<tbody>
<tr>
<td>N with values (%)</td>
<td>77 (81)</td>
<td>83 (87)</td>
<td>82 (86)</td>
<td>82 (86)</td>
<td>77 (81)</td>
<td>82 (86)</td>
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<tr>
<td>Detectable N ($)</td>
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<td>10 (12)</td>
<td>5 (6)</td>
<td>6 (7)</td>
<td>10 (11)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Elevated N (%)</td>
<td>74 (78)</td>
<td>84 (89)</td>
<td>80 (85)</td>
<td>82 (86)</td>
<td>77 (81)</td>
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</table>

This blood draw was not required per protocol.

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<table>
<thead>
<tr>
<th>Timepoint</th>
<th>AC#1</th>
<th>AC#2</th>
<th>AC#3</th>
<th>AC#4</th>
<th>PTL Wk7</th>
<th>PTL Wk9</th>
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<tbody>
<tr>
<td>N with values (%)</td>
<td>79 (81)</td>
<td>83 (87)</td>
<td>82 (86)</td>
<td>81 (85)</td>
<td>78 (82)</td>
<td>80 (85)</td>
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<td>Detectable N (%)</td>
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<td>6 (7)</td>
<td>10 (11)</td>
<td></td>
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<tr>
<td>Elevated N (%)</td>
<td>76 (80)</td>
<td>84 (89)</td>
<td>80 (85)</td>
<td>82 (86)</td>
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This blood draw was not required per protocol.
cTnI (P = 0.32 MSKCC, P = 0.41 DF/HCC) was not associated with the maximum LVEF decline from baseline. Finally, we examined the 9 (9%) patients with undetectable cTnI and no elevation in CRP throughout the study period. In this subgroup no patients developed CHF, 1 patient stopped treatment due to LVEF decline >15%, 2 patients had maximum LVEF decline of 10% to 15%, 1 patient had a maximum LVEF decline of <10%, 3 patients had maximum LVEF declines of <5%, and 2 patients had no LVEF declines.
Discussion

Although the mechanisms underlying anthracycline-induced cardiotoxicity such as the role of oxygen free-radicals have been well established (13–15), far less is known about T-related cardiotoxicity, in part due to the lack of relevant animal models and difficulty obtaining cardiac biopsies (16). It is also not known what role, if any, tyrosine kinase inhibitors, such as lapatinib, that target HER2 may play. However, it appears as though T-related cardiotoxicity is generally less severe and more readily reversible than damage from anthracyclines (1, 16). As noted, attempts at early detection of cardiotoxicity have focused on measuring LVEF, which has limited sensitivity (as an early predictor) because of the significant cardiac reserve in the normal heart. As a consequence, LVEF could represent a “trailing” rather than “leading” indicator of risk. Furthermore, measured changes in LVEF may reflect prior damage rather than prediction of additional dysfunction. A technical concern is that most measures of ventricular function are sensitive to loading conditions and only become abnormal after compensatory mechanisms have been overcome. In patients receiving sequential anthracycline-T-based treatment, age ≥50, lower post AC LVEF and requirement for antihypertensive medications have been proposed as associated risk factors for CHF (17) but a more refined predictive model is desirable to allow for personalized treatment decisions.

To the best of our knowledge this is the first prospective study examining detectable cTnIs as a possible predictive biomarker for cardiotoxicity in patients receiving both T and lapatinib following anthracycline-based chemotherapy. We have demonstrated that low levels of cTnI can be extremely commonly detected in the serum of patients undergoing adjuvant chemotherapy with ddAC—PTL. Overall, in this study, 67% of patients had at least one detectable cTnI measurement, 19 (20%) patients had a single detectable cTnI, and 45 (47%) had detectable cTnI at 2 or more timepoints. This appears to be higher than reports from studies predominantly using anthracyclines and high-dose chemotherapy (30%–34% of patients; refs. 18–23) but similar to preliminary data (58%–79% of patients; refs. 24 and 25) reported from studies of adjuvant chemotherapy for breast cancer, including some patients receiving ddAC and T. However, in the largest reported series of 251 patients receiving T, cTnI was only detected in 14% patients (26). The reasons for the higher incidence of detectable cTnIs in our study are unclear but could, in part, be related to the difference in cut-off values for detectable cTnIs (MSKCC ≥0.06 ng/mL, DF/HCC ≥0.04 ng/mL compared to ≥0.09 ng/mL in the previous report). In the study by Cardinale and colleagues, which included 123 (49%) patients with early breast cancer, T-induced cardiotoxicity (TIC; defined as absolute decrease in LVEF of >10% from baseline, associated with a decline below 50%) occurred more frequently in patients with cTnI increase than in those without (62% vs. 5%; P < 0.001; ref. 26). Importantly, cTnIs were retested for confirmation and were measured before and soon after each T infusion, although cross-study comparisons are challenging, in part because of the varying populations and methodologies employed.

In this study, abnormalities in cTnI occurred most frequently during PTL and did not persist during TL nor at 18-month follow-up (Fig. 4). The timing of detectable cTnIs appeared to precede the maximal decline in LVEF at month 6. For example after ddACx4, 10 (11%) patients had detectable cTnIs, rising to 41 (50%) patients following 4 weeks of PTL. This finding was similar to that reported by Cardinale and colleagues in which cTnI “positivity” was most prevalent soon after the first cycle of T (around 3–4 weeks of T; ref. 26). Most patients had normalization of cTnI after 3 months of anti-HER2 therapies, again very similar to Cardinale’s study (26). Therefore, the timing of these abnormalities suggests an added cardiac toxicity from anti-HER2 therapies after anthracyclines, although the impact of individual anti-HER2 agents, paclitaxel, and the relative contribution of anthracyclines cannot be determined. It is plausible that HER2 inhibition impeded the repair of cardiac myocyte damage from previous anthracycline exposure, which resolved by the 3rd month in most. Of note, in our study, 8% of patients had significant asymptomatic LVEF decline during anti-HER2 therapy compared to 11% of patients who had TIC while on adjuvant T in the Cardinale study (26). By traditional criteria, only 1 patient in this study (with no record or evidence of CHF) had a single elevated cTnI level (0.59 ng/mL) but this was immediately before her death from fulminant liver failure from reactivated Hepatitis B making this elevation uninterpretable.

In the diagnosis of acute coronary syndromes, a “positive” cTnI (>0.31 ng/mL) is often considered an all-or-nothing event, although mortality has been shown to increase proportionally to the absolute cTnI level (10). Because cTnI should be undetectable in the serum of healthy individuals, the reported finding of any detectable cTnIs is potentially clinically relevant. However, a challenge in the interpretation of these results is the observation that 4% of patients in the current study had a detectable cTnI before the administration of any chemotherapy, which was similar to the 3% (7 of 251 patients) reported by Cardinale and colleagues (26). It should be noted that approximately 80% of patients in the study by Cardinale and colleagues had received prior anthracyclines and patients in our study were chemotherapy-naive at baseline. However, the finding of detectable cTnI at baseline is surprising, given that patients with underlying cardiac dysfunction (unstable angina, myocardial infarction and CHF) were excluded from participation in our study. The mechanism for cTnI release in this setting is unclear, although it has been suggested that this may relate to subclinical myocardial injury and that anti-HER2 therapy could potentiate anthracycline-mediated myocyte damage (26). Because HER2 is expressed in cardiac myocytes, blockade of HER2 could lead to the loss of downstream HER2-mediated survival pathways (which have a physiologic role in protecting cardiac function and adapting to stress), leading to myocyte...
death and cTnI release (26, 27). In support of this, in both the current report and the recent study by Cardinale and colleagues, almost all patients with detectable cTnI were treated with anthracyclines (26). Furthermore, because cTnI is not routinely measured in asymptomatic patients, the prevalence of low levels of cTnI may be underappreciated in the general population, including in the post-surgical setting. Therefore, the relative contribution of individual therapies to these findings is unclear, but our results challenge the dogma that healthy individuals will not have detectable cTnI.

The link between troponins and anthracycline-induced cardiotoxicity is well established. In an animal model, cardiac troponins correlate with doxorubicin-induced cardiac injury (28). In children receiving doxorubicin, the magnitude of cardiac troponin rise correlates with left ventricular dilatation and wall thinning (29). Several studies have assessed the predictive role of troponins in adults, mainly in the setting of high-dose chemotherapy (18–21, 23). Cardinale and colleagues demonstrated that LVEF declines were transient in patients who had no rise in cTnI after high-dose chemotherapy compared to those who had at least 1 detectable cTnI (20). Subsequently, the same group demonstrated that a close correlation existed between the magnitude of cTnI rise and maximal LVEF depression ($r = -0.92$, $P < 0.0001$; ref. 19) In another study, patients who had persistently detectable cTnIs after high-dose chemotherapy also had a higher incidence of cardiac events compared to those whose cTnIs become undetectable (18).

Substantially less is known about these biomarkers in patients receiving adjuvant chemotherapy for breast cancer, although cardiac troponins can be detected in the serum of patients receiving similar doses of doxorubicin to that used in ACx4 and have correlated with diastolic dysfunction (21). In addition, there are preliminary reports of the use of troponin as a biomarker of T-induced cardiotoxicity, including data from a subgroup analysis in N9831, which support the possible role of cTnI in this setting (30). In contrast, no abnormal troponins were detected in a neoadjuvant study of T with anthracycline-based chemotherapy (31). However, we suggest that older studies may not have captured data on low-level troponin rises below the threshold for acute coronary syndrome, but which have subsequently been shown to be important (18–20).

Because of the role of renin–angiotensin system in the development of anthracycline-induced cardiotoxicity, there has been considerable interest in the preventative use of angiotensin-converting enzyme (ACE) inhibitors. These agents have been shown to slow the progression of left ventricular dysfunction in a variety of settings, including anthracycline-induced cardiomyopathy (32). In a randomized study, treatment with an ACE inhibitor prevented declines in LVEF following high-dose chemotherapy (32). In the study by Cardinale and colleagues, no patients with cTnI >0.07 ng/mL on an ACE inhibitor showed a decrease in LVEF >10% from baseline to below the lower limit of normal at 12 months, compared to 25 patients with cTnI >0.07 ng/mL on an ACE inhibitor showed no decrease in LVEF (43%; $P < 0.001$). However, in doxorubicin-treated children with left-ventricular dysfunction, the benefits of ACE inhibitors seem to be transient (33) and their role in the current setting has not been explored. Therefore, caution is required before routinely recommending these agents to patients with detectable cTnIs, treated with anthracyclines and anti-HER2 therapy, and a randomized trial is needed in this setting.

Despite the extensive abnormalities in the biomarkers described in our study, the maximum cTnI did not predict for the maximal recorded decline in LVEF. We believe there are several important factors to consider. Overall, the event rate was low (3% had CHF) and declines in LVEF were relatively uncommon, thereby limiting the statistical power. In addition, our results are confounded by the high drop-out rate, mainly due to PTL-related diarrhea, as many patients with toxicity did not continue biomarker assessment and many patients were noncompliant with blood draws up to 18 months. For example, although cTnI results are available for 92% of patients starting PTL, this declined to 23% patients at 18 month follow-up. In addition, this study correlated cTnI with LVEF measured by MUGA rather than pathologic changes within the myocardium. In contrast to MUGA, echocardiograms can detect diastolic dysfunction, which may be particularly important as an early sign of anthracycline cardiotoxicity (34). Therefore, the abnormalities in cTnI described may reflect subtle changes in diastolic function that are not detected by MUGA. We are also concerned that, the timing of the blood draws may have been suboptimal. Although not defined per protocol, standard institutional policy is to draw bloods before chemotherapy administration. Cardinale and colleagues have shown that cTnI peaks immediately after high-dose chemotherapy (20). Therefore, in our study cTnI drawn immediately before treatment may represent a nadir value and perhaps this biomarker would have been more predictive if drawn at an optimal posttreatment timepoint.

CRP is an acute phase reactant with a poorly defined physiologic role, which has been implicated in both anti-inflammatory and proinflammatory responses (11). Baseline CRP >3 mg/L has been associated with an increased risk of death from cancer and vascular disorders (35, 36). We incorporated CRP monitoring in this study in an attempt to refine risk stratification from cTnI alone (11). However, we observed no pattern within the extensive variations in CRP and we believe it lacks specificity for further investigation as a biomarker of cardiotoxicity, particularly given the confounding effect of surgery and infective and inflammatory processes. Nonetheless, this study defined a small subgroup of 9 (9%) patients who had undetectable cTnI and no elevation in CRP throughout the study period, none of whom developed CHF, thereby highlighting the possible negative predictive value of combining two biomarkers. This is consistent with previous reports suggesting that patients with consistently undetectable troponins may be at lower risk of cardiotoxicity (20, 23).
Several alternative biomarkers of cardiotoxicity have been proposed. Brain natriuretic peptide (BNP) has been extensively tested as a prognostic marker of CHF and has been examined for chemotherapy-related cardiotoxicity along with other biomarkers such as glycogen phosphorylase BB and heart-type fatty acid-binding protein (24, 37, 38). The epidermal growth factor neuregulin-1 (NRG-1) plays a critical role in the growth and survival of cardiac myocytes and is of particular interest since NRG-1 is a target of T (38). In CHF, NRG-1 correlates with disease severity and risk of death independently of BNP, although the combination of NRG-1 and BNP provides better risk stratification than either biomarker individually (38). This study adds weight to the argument that a single biomarker (such as cTnI) should not be used in isolation to stratify risk of cardiotoxicity and guide preventative strategies (such as ACE inhibitor use). In addition, there is growing evidence that echocardiograms and novel techniques such as Doppler echocardiography may provide the granularity to detect subtle changes in diastolic function, which is increasingly recognized as an important early event in the pathogenesis of drug-induced cardiotoxicity (34, 39). Ultimately, risk stratification may incorporate clinical parameters such as age and hypertension, functional cardiac assessment (LVEF), and several serum biomarkers in combination. Therefore, in an attempt to refine current predictive models of cardiotoxicity, we will prospectively assess cTnIs, BNP, and NRG-1 drawn immediately before and after anthracycline-T-based regimens with correlative LVEF monitoring in an upcoming study.

In summary, we have demonstrated that cTnI is commonly detected in the serum of patients receiving ddAC—PTL—TL. These abnormalities occur most commonly during PTL, highlighting the possible subclinical impact of anti-HER2 therapy after anthracyclines. In this study, neither cTnI nor CRP predicted the development of CHF or maximal LVEF decline, but definitive conclusions are limited by the low event rate, the high patient dropout (for other causes) and potentially the suboptimal timing of biomarker assessments. We plan to address these issues in a prospective study.

Disclosure of Potential Conflicts of Interest

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