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

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Statement of Translational Relevance.

While trastuzumab 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.

In a clinical trial, testing the feasibility of adding lapatinib to dose-dense AC followed by paclitaxel/trastuzumab, 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 trastuzumab, and possible under-appreciation 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.
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 pre-planned 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 cTnl levels did not correlate with LVEF declines. A detectable CRP was seen in 74/95 (78%) but did not correlate with LVEF declines.

Conclusions

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.
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).(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 trastuzumab was first appreciated in the metastatic setting.(1) As a surrogate for clinical CHF, the large phase III studies of adjuvant trastuzumab 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 pre-planned rules), but clinically asymptomatic, LVEF declines after conventionally scheduled doxorubicin and cyclophosphamide (AC).(2) In addition, of those eligible for trastuzumab, 31.4% discontinued therapy prior to 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 (dose dense [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 trastuzumab
was associated with an overall CHF rate of 1.4% (1/70 patients).(6) Taken together, these findings suggest that dd anthracycline-based chemotherapy incorporating trastuzumab 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, the development of effective cardiac biomarkers is desirable.

Cardiac Troponin I (cTnI) 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 PT and lapatinib (L), we included a pre-planned analysis of both cTnI and CRP as early biomarkers of cardiotoxicity.
**Materials and Methods**

As previously described eligible patients had early breast cancer which overexpressed HER-2/neu regardless of nodal status or tumor size. 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 time-points (figure 1). Although not specified by the protocol, it is standard practice at our institutions for research blood tests to be drawn immediately prior to 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 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., San Francisco, CA) with a low end sensitivity of 0.06 ng/ml (MSKCC) and 0.04ng/ml (DF/HCC). Per institutional policy a cTnI reading of ≥0.31ng/ml was considered elevated in the setting of acute coronary syndrome, but any detectable cTnI (≥0.06 ng/ml [MSKCC] and ≥0.04ng/ml [DF/HCC]) was considered abnormal for the purposes of the study. The CRP was measured by particle-enhanced immunoturbidimetry, with a lower limit detection of 0.02 mg/l (Beckman-Colter).
Diagnostics, Brea, CA). Per institutional policy elevated CRP was defined as ≥0.8mg/dl (MSKCC) and ≥0.3mg/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 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→TL, 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). Upon completion of ddAC (and prior to PTL week 1) 87 (92%) of patients had cTnI measured, of which 10 (11%) were detectable. During PTL 67-92% of blood draws occurred per protocol and detectable cTnIs were common (figures 3A, 4). The timing of these detectable cTnIs appeared to precede maximum decline in LVEF as shown in figures 4A and 4B, which are time-series graphs, showing mean LVEF and cTnI with standard errors at protocol-defined time-points. For example, prior to 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 time-points, and 19 had detectable cTnIs at 3 time-points. 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 (figure
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. Upon completion of ddAC, (prior to 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 0.08ng/ml, DF/HCC) prior to AC#2. Repeat cTnI prior to AC#3, AC#4, PTL weeks #1 and #3 were undetectable. Her CRP levels were elevated at baseline (3.7mg/dl, DF/HCC) and peaked prior to AC#2 (45.1mg/dl) with subsequent values of 0.8mg/dl, 0.9mg/dl 2.5mg/dl and 28.4mg/dl. However, she had no blood draw immediately prior to 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 prior to week 3 of PTL. She had multiple elevations in CRP, including baseline (1.43mg/dl, MSKCC) and month 6 (1.1mg/dl), to a maximum of 3.08mg/dl prior to 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 month12). Her cTnI was undetectable throughout treatment until immediately prior to 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 0.97mg/dl (MSKCC) prior to 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. Prior to 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 (0.06ng/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, two 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 (0.15ng/ml and 0.12ng/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 ng/ml, 0.16 ng/ml, 0.13 ng/ml, 0.06 ng/ml, 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 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 10-15%, 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 trastuzumab-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, like lapatinib, that target HER2 may play. However, it appears as though trastuzumab-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-trastuzumab-based treatment, age ≥50, lower post AC LVEF and requirement for anti-hypertensive 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 trastuzumab 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 time-points. This appears to be higher than reports from studies predominantly using anthracyclines and high dose chemotherapy (30-34% of patients),(18-23) but similar to preliminary data (58-79% of patients)(24, 25) reported from studies of adjuvant chemotherapy for breast cancer, including some patients receiving ddAC and trastuzumab. However, in the largest reported series of 251 patients receiving trastuzumab, 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 ≥0.06ng/ml, DF/HCC ≥0.04ng/ml compared to ≥0.09ng/ml in the previous report). In the study by Cardinale et al, which included 123 (49%) patients with early breast cancer, trastuzumab 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). Importantly, cTnIs were retested for confirmation and were measured before and soon after each trastuzumab infusion, although cross-study comparisons are challenging, in part because of the varying populations and methodologies employed.

In the current study, abnormalities in cTnI occurred most frequently during PTL and did not persist during TL nor at 18-month follow-up (figure 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 et al in which cTnI “positivity” was most prevalent soon after the first cycle of trastuzumab (around 3-4 weeks of trastuzumab) (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 trastuzumab in the Cardinale study (26). By traditional criteria only 1 patient in the current study (with no record or evidence of CHF) had a single elevated cTnI level (0.59ng/ml) but this was
immediately prior to her death from fulminant liver failure from reactivated Hepatitis B making this elevation uninterpretable.

In the diagnosis of acute coronary syndromes, a “positive” cTnI (>0.31ng/ml) is often considered an all-or-nothing event, although mortality has been shown to increase proportionally to the absolute cTnI level.(10) Since 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 prior to the administration of any chemotherapy, which was similar to the 3% (7 of 251 patients) reported by Cardinale et al (26). It should be noted that approximately 80% of patients in the study by Cardinale et al. had received prior anthracyclines and patients in our study were chemotherapy-naïve 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) Since HER2 is expressed in cardiac myocytes, blockade of HER2 could lead to the loss of downstream HER2–mediated survival pathways (which have a physiological 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 et al, almost all patients with detectable cTnI were treated with anthracyclines.(26)
Furthermore, since 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 et al 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 one 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).(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 trastuzumab-induced cardiotoxicity, including data from a subgroup analysis in N9831, which support the possible role of cTnl in this setting. (30) In contrast, no abnormal troponins were detected in a neoadjuvant study of trastuzumab 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 function following high dose chemotherapy. (32) In the study by Cardinale et al, no patients with cTnl >0.07ng/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 controls (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 non-compliant 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 pathological 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. 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 prior to chemotherapy administration. Cardinale et al have shown that cTnI peaks immediately after high dose chemotherapy. 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 post-treatment time-point.

CRP is an acute phase reactant with a poorly defined physiological role, which has been implicated in both anti-inflammatory and proinflammatory responses.
CRP >3mg/l has been associated with an increased risk of death from cancer and vascular disorders. We incorporated CRP monitoring in the current study in an attempt to refine risk-stratification from cTnI alone. 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, the current 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.

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. 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 trastuzumab. 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. The current 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. 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-trastuzumab 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.
Figure Legends

Figure 1 Study Schema and timing of blood draws

Table 1. Baseline Characteristics of Patients (N=95)

Table 2. Cardiac troponin I and CRP measured per protocol

Figure 2. LVEF Results: Median Follow-up 22 months
Footnote; individual patient results shown in different colors

Figure 3. Detectable cTnI and CRP values for all patients (n=95)
   A. cTnI
   B. CRP

Footnote; dd AC = dose dense doxorubicin and cyclophosphamide. PTL = Paclitaxel Trastuzumab and Lapatinib, TL = Trastuzumab and Lapatinib

Figure 4. Maximum cTnI rise appears to precede maximum decline in LVEF.
   A. MSKCC.
   B. DF/HCC

Footnote – Time series graph of left ventricular ejection fraction and cTnI showing mean values and standard errors at time-points defined per protocol. The figure for DF/HCC is truncated at month 9 as only 1 patient completed 18 month assessment.
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<tr>
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<td>17</td>
<td>5</td>
</tr>
<tr>
<td>No. of involved nodes</td>
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</tr>
<tr>
<td>0</td>
<td>15</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>1-3</td>
<td>39</td>
<td>41</td>
<td>12</td>
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<td>4-9</td>
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<tr>
<td>10+</td>
<td>11</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Unknown (neoadjuvant)</td>
<td>16</td>
<td>17</td>
<td>5</td>
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<td>56</td>
<td>16</td>
<td>52</td>
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<td>58</td>
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<tr>
<td>Negative</td>
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<td>44</td>
<td>15</td>
<td>48</td>
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<td>55</td>
<td>16</td>
<td>52</td>
<td>36</td>
<td>56</td>
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</table>

| Pre-existing Hypertension | 17 | 18 | 5  | 16 | 12 | 19 |

| Breast/chest radiation | 74 | 78 | 28 | 46 |  |  |
| Left sided radiation   | 37/74 | 50 | 14/28 | 50 | 23/46 | 50 |
| Right sided radiation  | 36/74 | 49 | 14/28 | 50 | 22/46 | 48 |
| Bilateral radiation    | 1/74 | 1  |  | 1/46 | 2  |  |

*Includes 1 patient with prior mastectomy and subsequent ipsilateral recurrence.

Figures rounded to the nearest percentage
Table 2. Cardiac troponin and CRP measured per protocol

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<tr>
<th></th>
<th>AC #1</th>
<th>AC #2</th>
<th>AC #3</th>
<th>AC #4</th>
<th>PTL Wk 1</th>
<th>PTL Wk 3</th>
<th>PTL Wk 5</th>
<th>PTL Wk 7</th>
<th>PTL Wk9</th>
<th>PTL Wk 11</th>
<th>Mth 5*</th>
<th>Mth 6</th>
<th>Mth 9</th>
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<tr>
<td><strong>Cardiac troponin I</strong></td>
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<tr>
<td>N with values (%)</td>
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<td>83 (87)</td>
<td>82 (86)</td>
<td>81 (85)</td>
<td>87 (92)</td>
<td>77 (81)</td>
<td>82 (86)</td>
<td>75 (79)</td>
<td>70 (74)</td>
<td>64 (67)</td>
<td>23 (24)</td>
<td>60 (63)</td>
<td>62 (65)</td>
<td>22 (23)</td>
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<tr>
<td>Detectable N (%)</td>
<td>3 (4)</td>
<td>10 (12)</td>
<td>5 (6)</td>
<td>6 (7)</td>
<td>10 (11)</td>
<td>19 (25)</td>
<td>41 (50)</td>
<td>39 (52)</td>
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<td>3 (5)</td>
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<td>1 (2)</td>
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<td><strong>C-Reactive Protein</strong></td>
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<td></td>
<td></td>
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<tr>
<td>N with values (%)</td>
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<td>84 (88)</td>
<td>80 (84)</td>
<td>81 (85)</td>
<td>89 (94)</td>
<td>82 (86)</td>
<td>83 (87)</td>
<td>76 (80)</td>
<td>73 (77)</td>
<td>68 (72)</td>
<td>20 (21)</td>
<td>58 (61)</td>
<td>62 (65)</td>
<td>28 (29)</td>
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<td>Elevated N (%)</td>
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<td>40 (48)</td>
<td>32 (40)</td>
<td>35 (43)</td>
<td>42 (47)</td>
<td>38 (46)</td>
<td>46 (55)</td>
<td>36 (47)</td>
<td>33 (45)</td>
<td>26 (38)</td>
<td>3 (15)</td>
<td>27 (47)</td>
<td>25 (40)</td>
<td>17 (61)</td>
</tr>
</tbody>
</table>

*This blood draw was not required per protocol

Table showing absolute number of patients with detectable cTnI (≥0.06 ng/ml [MSKCC] and ≥0.04ng/ml [DF/HCC]) and elevated CRP (≥0.8mg/dl [MSKCC] and ≥0.3mg/dl [DF/HCC]) at each time-point per protocol.
References

**Figure 1**

- **Doxorubicin**
  * 60 mg/m² q2w

- **Paclitaxel**
  * 80 mg/m² weekly

- **Cyclophosphamide**
  * 600 mg/m² q2w

- **Trastuzumab**
  * 2 mg/kg weekly
  * 6 mg/kg q3w

- **Lapatinib**
  * 1000 mg/day

- **Radiation** given concurrently with trastuzumab

- **MUGA scans** at 0, 2, 6, 9 and 18 months

- **Troponin I and C-Reactive Protein** measured every 2 weeks during chemotherapy and at 6, 9 and 18 months

- *Pegfilgrastim 6 mg is given on day 2 after chemotherapy*
Figure 2

N=95
Median 68%
Range 52-81%

N=93
Median 69%
Range 47-81%

N=89
Median 65%
Range 24-80%

N=76
Median 65%
Range 45-76%

N=30
Median 65%
Range 30-78%

LVEF

0 2 6 9 18

Months
Figure 3A

![Graph showing TnI levels over months for different conditions: dd AC, PTL, and TL.](image-url)
Figure 4A

![Graph showing LVEF and Troponin levels over time.](image-url)
Troponin I and C-Reactive Protein are Commonly Detected in Patients with Breast Cancer Treated with Dose-Dense Chemotherapy Incorporating Trastuzumab and Lapatinib.


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