Early Biochemical, Oxidative-Stress, Inflammatory, and Clinical Features of Anthracycline-Associated Cardiac Injury and Dysfunction in Patients with Breast Cancer: A Prospective Clinical Toxicology Study

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Abstract: *Background*: Anthracycline-associated cardiac injury remains an important limitation of breast-cancer chemotherapy. This study characterized early cardiac, oxidative-stress, antioxidant, inflammatory, and clinical findings observed during the same post-cycle-two assessment window.

Methods: This prospective clinical toxicology study included 150 women with breast cancer receiving anthracycline-containing chemotherapy. Measurements were obtained at baseline and after the second cycle. A composite early cardiac injury/cancer therapy-related cardiac dysfunction (CTRCD) classification comprised biomarker-defined myocardial injury without clinical dysfunction (n=10) and clinical CTRCD (n=32). Because post-cycle-two biomarkers and cardiac status were assessed concurrently, these biomarkers were analyzed descriptively and were not modeled as temporal predictors or evaluated by receiver operating characteristic analysis. Univariable logistic regression was restricted to baseline clinical factors.

Results: Forty-two participants met the composite classification and 108 did not. Baseline left ventricular ejection fraction (LVEF) and most baseline biomarkers were comparable, although baseline C-reactive protein (CRP) was higher in the composite group (8.56±5.92 vs 5.94±3.93 mg/L; P=0.010). After cycle two, the composite group had larger increases in troponin, N-terminal pro-B-type natriuretic peptide (NT-proBNP), creatine kinase-MB, lactate dehydrogenase, malondialdehyde, CRP, interleukin-6, and tumor necrosis factor-α, together with greater declines in LVEF, superoxide dismutase, glutathione peroxidase, catalase, and reduced glutathione (all change-score comparisons P<0.001). Hypertension (OR 2.87, 95% CI 1.36–6.06) and baseline LVEF <60% (OR 2.53, 95% CI 1.17–5.50) were associated with the composite classification in univariable analyses.

Conclusions: Early anthracycline-associated cardiac injury and dysfunction were accompanied by a concurrent multi-pathway profile of myocardial injury, wall stress, oxidative imbalance, antioxidant depletion, and inflammation. These findings support hypothesis-generating, multimodal surveillance research, but they do not establish temporal prediction or independent diagnostic performance for biomarkers measured during the same assessment window.

Keywords: Anthracyclines, Breast cancer, Cancer therapy-related cardiac dysfunction, Cardiotoxicity, Inflammation, Oxidative stress.

INTRODUCTION

Breast cancer is the most frequently diagnosed malignancy among women worldwide and remains a leading cause of cancer-related mortality. GLOBOCAN 2022 estimated more than 2.3 million new breast-cancer diagnoses globally, while continuing improvements in treatment and survivorship have increased the importance of preventing and managing long-term treatment toxicity [1,2].

Anthracyclines, including doxorubicin and epirubicin, remain important components of chemotherapy for early-stage and locally advanced breast cancer. Their use is limited by dose-related and patient-dependent cardiovascular toxicity, which may present as biochemical myocardial injury, asymptomatic left ventricular dysfunction, or symptomatic heart failure during treatment or later in survivorship [3-5]. Considerable interindividual variability at similar cumulative exposure suggests that baseline cardiovascular vulnerability and treatment-emergent biological responses both contribute to susceptibility [4,5].

Transthoracic echocardiography, particularly serial assessment of left ventricular ejection fraction (LVEF), remains central to surveillance. However, LVEF has limited sensitivity for early myocardial injury and may decline only after clinically important damage has occurred. Contemporary cardio-oncology guidance therefore combines baseline cardiovascular risk assessment, imaging, symptoms, and cardiac biomarkers, while emphasizing consistent and reproducible definitions of cancer therapy-related cardiac dysfunction (CTRCD) [4,6,7].

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Cardiac troponins and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are the most established circulating markers in this setting. Troponin reflects cardiomyocyte injury, whereas NT-proBNP reflects myocardial wall stress and hemodynamic load. Their interpretation depends on the assay, reference limits, sampling time, baseline concentration, and whether the measurement precedes or coincides with the cardiac outcome being evaluated [8-12]. Creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) may provide additional, although less specific, biochemical context.

Oxidative stress is a central mechanism of anthracycline-associated myocardial injury. Redox cycling, iron-dependent reactions, mitochondrial dysfunction, topoisomerase-IIβ-related DNA damage, calcium dysregulation, and cell-death pathways can increase reactive oxygen species and lipid peroxidation while depleting endogenous antioxidant defenses [13-16]. Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, and reduced glutathione (GSH) can therefore characterize the balance between oxidative damage and antioxidant capacity, although these assays are not yet standardized for routine cardio-oncology care.

Inflammatory signaling may amplify oxidative injury, endothelial dysfunction, and adverse myocardial remodeling. C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) have been investigated as treatment-emergent markers, but their clinical specificity is limited and baseline inflammation can confound change-based comparisons [15,16]. Accordingly, concurrent biomarker differences should not be interpreted as proof of temporal prediction without an earlier measurement that clearly precedes a later, independently defined outcome.

Evidence integrating cardiac, oxidative-stress, antioxidant, and inflammatory pathways in Middle Eastern breast-cancer populations remains limited. This prospective study therefore aimed to characterize baseline and post-cycle-two changes across these pathways and to examine baseline clinical factors associated with a composite early cardiac injury/CTRCD classification. The analysis was deliberately framed as concurrent early detection and phenotypic characterization, rather than prediction, because biomarkers and cardiac status were assessed within the same post-cycle-two window.

METHODS

Study Design and Setting

This prospective clinical toxicology study was conducted among women receiving anthracycline-containing chemotherapy in Baghdad, Iraq, from January 2024 through March 2025. It was designed and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations [17]. Participants were followed from a pretreatment baseline assessment to a prespecified evaluation after completion of the second chemotherapy cycle. The objective was to characterize concurrent early cardiac, biochemical, oxidative-stress, antioxidant, inflammatory, and clinical findings and to examine associations with baseline clinical factors.

Study Population

Women aged 18 years or older with histologically confirmed breast cancer who were scheduled to receive an anthracycline-containing regimen were screened consecutively. Eligibility required a planned doxorubicin- or epirubicin-containing regimen, a complete baseline clinical and cardiac assessment, and complete post-cycle-two follow-up. Exclusion criteria were previous anthracycline exposure, symptomatic heart failure, recent acute coronary syndrome, severe valvular heart disease, uncontrolled hypertension, severe renal or hepatic impairment, active systemic inflammatory disease, pregnancy, incomplete biomarker data, or incomplete follow-up.

The protocol was approved by the Scientific Research Ethics Committee of the Medical City Complex, Baghdad, Iraq (approval no. EC24-69-20). Written informed consent was obtained before enrollment. Participants were assigned anonymized study codes for data collection and analysis.

Sample Size Determination

The sample size was calculated in G*Power version 3.1 for a two-sided independent-groups comparison of the prespecified change in NT-proBNP. The calculation assumed a standardized mean difference of 0.52, a composite-outcome-to-no-outcome allocation ratio of 0.40, α=0.05, and 80% power, yielding a minimum of 145 participants. The target was increased to 150 to allow for approximately 3% incomplete or non-evaluable follow-up [18]. The analyzed cohort comprised 108 participants without and 42 with the composite early cardiac injury/CTRCD classification.

Clinical and Treatment Variables

Baseline demographic, clinical, oncological, and treatment variables were recorded using a structured form. Variables included age, body mass index, menopausal status, hypertension, diabetes mellitus, cancer stage, human epidermal growth factor receptor 2 (HER2) status, planned trastuzumab therapy, baseline LVEF, anthracycline regimen, and planned totalcourse anthracycline exposure. Regimens included doxorubicin plus cyclophosphamide (AC), fluorouracildoxorubicincyclophosphamide (FAC), fluorouracilepirubicincyclophosphamide (FEC), and dosedense AC.

Planned totalcourse anthracycline exposure was recorded from the pretreatment regimen and expressed in doxorubicinequivalent mg/m². Doxorubicin doses were multiplied by 1.00 and epirubicin doses by 0.67 before summation [20]. Hypertension and diabetes were defined from documented medical history, active pharmacological treatment, or physician diagnosis. HER2 status and trastuzumab planning were abstracted from oncology records.

Cardiac Assessment and Operational Classification

Transthoracic echocardiography was performed at baseline and after the second cycle using a Philips EPIQ 7C system with a phased-array transducer. LVEF was calculated by the biplane Simpson method from apical two- and four-chamber views by the same experienced cardiologist, who was unaware of the laboratory results. The post-cycle-two echocardiogram was completed within 72 hours of the blood draw.

The study used two mutually exclusive categories within a composite early cardiac injury/CTRCD classification. The terminology distinguishes biochemical myocardial injury from clinically defined ventricular dysfunction and avoids treating referral or chemotherapy delay as diagnostic criteria.

Biomarker-defined myocardial injury was defined as an absolute increase in cardiac troponin I of ≥0.02 ng/mL (20 ng/L) and/or an increase in NT-proBNP of ≥100 pg/mL from baseline, without new cardiac symptoms and without meeting the clinical CTRCD criterion.

Clinical CTRCD was defined as an absolute LVEF reduction of ≥10 percentage points from baseline to <50%, a new LVEF <40%, or new heart-failure symptoms with cardiologist-confirmed anthracycline-associated cardiac dysfunction. Cardiology referral and chemotherapy dose modification were recorded as consequences and were not used by themselves to define CTRCD.

Two investigators independently reviewed baseline and post-cycle-two echocardiography, biomarker results, symptoms, and clinical records. Disagreements were resolved by consensus with the treating cardiologist.

Participants meeting either category were included in the composite classification. The thresholds were prespecified for the study and interpreted in the context of contemporary IC-OS/ESC definitions [4,19]. Because the defining biomarkers and cardiac status were measured in the same post-cycle-two window, analyses of post-cycle-two biomarkers were considered concurrent characterization rather than temporal prediction.

Blood Sample Collection and Processing

Peripheral venous blood was obtained at baseline before the first anthracycline dose and 24±4 hours after completion of the anthracycline infusion in cycle two. Samples were processed within two hours. Serum and EDTA-plasma aliquots were prepared as required, stored at −80°C, and protected from repeated freeze–thaw cycles. The post-cycle-two blood draw and echocardiogram therefore represented the same early assessment window rather than temporally separated predictor and outcome measurements.

Laboratory Assessments

Cardiac biomarkers included cardiac troponin I, NT-proBNP, CK-MB, and LDH. Oxidative-stress and antioxidant markers included MDA, SOD, GPx, catalase, and GSH. Inflammatory biomarkers included CRP, IL-6, and TNF-α. Each marker was measured at baseline and after cycle two, and change was calculated as the post-cycle-two value minus baseline for markers expected to rise. For LVEF and antioxidant markers, decline was calculated as baseline minus post-cycle-two value so that a larger positive value represented a larger adverse change.

Assay manufacturer, platform, specimen matrix, analytical sensitivity, measuring range, and precision are reported in Supplementary Table **S1**. Internal quality control, calibration, duplicate analysis of selected samples, and rejection of visibly hemolyzed or insufficient samples were applied according to the assay instructions.

Routine Laboratory and Inflammatory Indices

Baseline routine laboratory variables included hemoglobin, serum creatinine, alanine aminotransferase (ALT), albumin, neutrophil count, lymphocyte count, and platelet count. The neutrophil-to-lymphocyte ratio was calculated as absolute neutrophils divided by absolute lymphocytes; the platelet-to-lymphocyte ratio as platelets divided by absolute lymphocytes; and the systemic immune-inflammation index as platelets × neutrophils / lymphocytes.

Clinical Consequences

Clinical consequences recorded during early follow-up were new or worsening cardiac symptoms, cardiology referral, and chemotherapy delay due to cardiac concern. Symptoms included dyspnea, palpitations, chest discomfort, edema, or other clinician-documented features suggestive of cardiac dysfunction. Referral was defined as specialist cardiovascular review prompted by symptoms, an LVEF change, or an abnormal cardiac biomarker. Dose delay was defined as postponement of the planned chemotherapy administration because of suspected or confirmed cardiac toxicity.

Statistical Analysis

Analyses were performed using IBM SPSS Statistics version 29.0. Distributional assumptions were assessed with the Shapiro-Wilk test, histograms, and Q-Q plots. Continuous variables are summarized as mean±standard deviation or median and interquartile range, as appropriate; categorical variables as n (%). Independent-samples t tests or Mann-Whitney U tests were used for between-group comparisons, paired t tests or Wilcoxon signed-rank tests for within-participant changes, and chi-square or Fisher exact tests for categorical variables.

The principal comparison was between participants with and without the composite early cardiac injury/CTRCD classification. Cardiac biomarker comparisons were explicitly descriptive because troponin and NT-proBNP contributed to biomarker-defined myocardial injury in 10 participants. CRP change was also interpreted descriptively because baseline CRP differed between groups. No post-cycle-two biomarker was entered as a predictor, and receiver operating characteristic analyses and the internally derived combined score were removed to avoid incorporation bias and same-time-point prediction claims.

Univariable logistic regression evaluated only baseline or treatmentexposure factors specified before the postcycletwo classification: age ≥55 years, hypertension, diabetes, planned totalcourse doxorubicinequivalent dose ≥300 mg/m², and baseline LVEF <60%. Odds ratios (ORs), 95% confidence intervals (CIs), and P values are reported. With 42 composite events and five candidate baseline factors, the analyses were considered exploratory; no multivariable model was fitted.

For Figure **1**, standardized between-group differences in adverse change were calculated as Hedges g with 95% CIs from the group means, standard deviations, and sample sizes reported in Tables **2** and **3**. Positive values indicate a larger adverse change in the composite group. Because numerous biomarkers were examined, no formal multiplicity correction was applied; P values are unadjusted and are interpreted together with effect magnitude, biological coherence, and the exploratory nature of the study. Statistical significance was defined as a two-sided P<0.05.

RESULTS

Patient Characteristics and Treatment Profile

A total of 150 women receiving anthracyclinecontaining chemotherapy were analyzed. Fortytwo participants met the composite early cardiac injury/CTRCD classification and 108 did not. Baseline demographic, clinical, and treatment characteristics are shown in Table **1**. Age, body mass index, mean baseline LVEF, planned totalcourse doxorubicin-equivalent dose, menopausal status, diabetes, cancer stage, HER2 status, planned trastuzumab therapy, and regimen distribution did not differ significantly between groups. Hypertension was more frequent in the composite group (47.6% vs 24.1%; P=0.009).

Cardiac Biomarker and LVEF Changes

Baseline troponin, NT-proBNP, CK-MB, LDH, and LVEF were not statistically different between groups (Table **2**). At the post-cycle-two assessment, the composite group had higher troponin, NT-proBNP, CK-MB, and LDH values, a lower mean LVEF, and larger adverse change scores. Mean LVEF decline was 9.1±3.7 percentage points in the composite group and 2.3±2.1 percentage points in the no-composite group (P<0.001). Because troponin and NT-proBNP contributed to classification for 10 participants and were measured during the same assessment window,

Table 1: Baseline Demographic, Clinical, and Treatment Characteristics According to Composite Early Cardiac Injury/CTRCD Classification

Variable	Total (N=150)	No composite classification (n=108)	Composite classification (n=42)	P value
Age, years	50.2 ± 9.3	49.8 ± 9.7	51.3 ± 8.2	0.337
Body mass index, kg/m²	28.30 ± 4.11	28.19 ± 4.27	28.56 ± 3.72	0.603
Baseline LVEF, %	63.0 ± 3.9	63.3 ± 3.7	62.4 ± 4.4	0.238
Planned totalcourse doxorubicinequivalent dose, mg/m²	256.4 ± 55.4	252.0 ± 53.1	267.8 ± 60.1	0.140
Menopausal status				0.449
Postmenopausal	102 (68.0)	71 (65.7)	31 (73.8)	
Premenopausal	48 (32.0)	37 (34.3)	11 (26.2)	
Hypertension				0.009
No	104 (69.3)	82 (75.9)	22 (52.4)	
Yes	46 (30.7)	26 (24.1)	20 (47.6)	
Diabetes mellitus				0.234
Yes	16 (10.7)	9 (8.3)	7 (16.7)	
No	134 (89.3)	99 (91.7)	35 (83.3)	
Cancer stage				0.661
I	46 (30.7)	34 (31.5)	12 (28.6)	
II	70 (46.7)	48 (44.4)	22 (52.4)	
III	34 (22.7)	26 (24.1)	8 (19.0)	
HER2-positive disease				0.933
No	99 (66.0)	72 (66.7)	27 (64.3)	
Yes	51 (34.0)	36 (33.3)	15 (35.7)	
Trastuzumab planned				0.464
No	125 (83.3)	92 (85.2)	33 (78.6)	
Yes	25 (16.7)	16 (14.8)	9 (21.4)	
Anthracycline regimen				0.639
AC	72 (48.0)	53 (49.1)	19 (45.2)	
Dose-dense AC	28 (18.7)	21 (19.4)	7 (16.7)	
FAC	27 (18.0)	20 (18.5)	7 (16.7)	
FEC	23 (15.3)	14 (13.0)	9 (21.4)	

Data are mean ± standard deviation or n (%). AC, doxorubicin plus cyclophosphamide; FAC, fluorouracil, doxorubicin, and cyclophosphamide; FEC, fluorouracil, epirubicin, and cyclophosphamide; HER2, human epidermal growth factor receptor 2; LVEF, left ventricular ejection fraction. P values are unadjusted.

Table 2: Cardiac biomarker and LVEF values and changes according to composite classification

Variable	Total(N=150)	No composite classification (n=108)	Composite Classification (n=42)	P value
Troponin, ng/mL, baseline	0.0086 ± 0.0029	0.0085 ± 0.0030	0.0088 ± 0.0024	0.505
Troponin, ng/mL, after cycle 2	0.0191 ± 0.0113	0.0140 ± 0.0061	0.0320 ± 0.0113	<0.001
Troponin increase, ng/mL	0.0105 ± 0.0108	0.0056 ± 0.0051	0.0232 ± 0.0115	<0.001
NT-proBNP, pg/mL, baseline	82.3 ± 52.2	76.6 ± 46.0	96.8 ± 64.0	0.067
NT-proBNP, pg/mL, after cycle 2	159.4 ± 93.9	117.1 ± 57.2	268.2 ± 81.5	<0.001
NT-proBNP increase, pg/mL	77.1 ± 74.4	40.4 ± 33.7	171.4 ± 67.1	<0.001
CK-MB, U/L, baseline	14.9 ± 3.9	14.6 ± 3.6	15.8 ± 4.6	0.133
CK-MB, U/L, after cycle 2	18.6 ± 6.1	16.4 ± 4.2	24.5 ± 6.4	<0.001
CK-MB increase, U/L	3.7 ± 4.2	1.8 ± 2.2	8.8 ± 4.1	<0.001
LDH, U/L, baseline	202.0 ± 36.9	202.5 ± 38.7	200.6 ± 32.1	0.757
LDH, U/L, after cycle 2	234.0 ± 52.4	225.8 ± 50.0	255.3 ± 53.1	0.003
LDH increase, U/L	32.1 ± 32.4	23.3 ± 25.6	54.7 ± 37.0	<0.001
LVEF, %, baseline	63.0 ± 3.9	63.3 ± 3.7	62.4 ± 4.4	0.238
LVEF, %, after cycle 2	58.8 ± 5.9	61.0 ± 4.2	53.3 ± 5.8	<0.001
LVEF decline, percentage points	4.2 ± 4.0	2.3 ± 2.1	9.1 ± 3.7	<0.001

Data are mean ± standard deviation. Change denotes post-cycle-two minus baseline; LVEF decline denotes baseline minus post-cycle-two. CK-MB, creatine kinase-MB; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide. P values are descriptive and unadjusted. Troponin and NT-proBNP contributed to biomarker-defined myocardial injury for 10 participants and must not be interpreted as independent predictors or diagnostic-performance estimates.

these differences describe the phenotype and do not represent independent prediction or diagnostic accuracy.

Oxidative-Stress, Antioxidant, and Inflammatory Changes

Baseline MDA, SOD, GPx, catalase, GSH, IL-6, and TNF-α were comparable between groups. Baseline CRP was higher in the composite group (8.56±5.92 vs 5.94±3.93 mg/L; P=0.010). After cycle two, the composite group had a larger MDA increase and larger declines in SOD, GPx, catalase, and GSH. CRP, IL-6, and TNF-α increases were also larger (all change-score comparisons P<0.001; Table **3**). The CRP change comparison is descriptive and unadjusted for its baseline imbalance.

Baseline Routine Laboratory and Inflammatory Indices

Hemoglobin, creatinine, ALT, albumin, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and systemic immune-inflammation index did not differ significantly at baseline (Table **4**).

Composite Classification and Clinical Consequences

Among the 42 participants in the composite group, 10 had biomarker-defined myocardial injury without clinical CTRCD and 32 met the clinical CTRCD criterion. Cardiac symptoms occurred in 33.3% of the composite group versus 1.9% of the no-composite group; cardiology referral in 61.9% versus 6.5%; and chemotherapy delay due to cardiac concern in 28.6% versus 5.6% (all P<0.001; Table **5**). These outcomes were recorded as consequences and not as stand-alone defining criteria.

Baseline Clinical Associations

In univariable analyses, hypertension was associated with the composite classification (OR 2.87, 95% CI 1.36–6.06; P=0.006), as was baseline LVEF <60% (OR 2.53, 95% CI 1.17–5.50; P=0.021). Age ≥55

Table 3: Oxidative-Stress, Antioxidant, and Inflammatory Biomarker Values and Changes

Variable	Total (N=150)	No composite classification (n=108)	Composite Classification (n=42)	P value
MDA, nmol/mL, baseline	2.76 ± 0.64	2.74 ± 0.66	2.80 ± 0.61	0.592
MDA increase, nmol/mL	0.62 ± 0.53	0.37 ± 0.28	1.25 ± 0.51	<0.001
SOD, U/mL, baseline	66.7 ± 11.8	67.2 ± 11.6	65.5 ± 12.3	0.461
SOD decline, U/mL	6.6 ± 5.5	4.4 ± 3.8	12.2 ± 5.3	<0.001
GPx, U/mL, baseline	46.1 ± 8.9	45.8 ± 9.0	46.7 ± 8.5	0.579
GPx decline, U/mL	4.2 ± 4.2	2.6 ± 3.1	8.1 ± 4.2	<0.001
Catalase, U/mL, baseline	55.9 ± 10.2	56.0 ± 10.0	55.5 ± 10.6	0.771
Catalase decline, U/mL	5.1 ± 5.4	3.0 ± 4.3	10.5 ± 4.3	<0.001
GSH, µmol/L, baseline	6.07 ± 1.05	6.00 ± 1.02	6.26 ± 1.12	0.188
GSH decline, µmol/L	0.70 ± 0.66	0.41 ± 0.36	1.44 ± 0.66	<0.001
CRP, mg/L, baseline	6.67 ± 4.71	5.94 ± 3.93	8.56 ± 5.92	0.010
CRP increase, mg/L	3.65 ± 3.54	2.36 ± 2.24	6.96 ± 4.11	<0.001
IL-6, pg/mL, baseline	4.69 ± 2.53	4.45 ± 2.42	5.30 ± 2.74	0.085
IL-6 increase, pg/mL	2.56 ± 2.46	1.50 ± 1.31	5.28 ± 2.64	<0.001
TNF-α, pg/mL, baseline	8.73 ± 4.49	8.57 ± 4.27	9.15 ± 5.04	0.510
TNF-α increase, pg/mL	2.23 ± 2.28	1.21 ± 1.34	4.86 ± 2.07	<0.001

Data are mean ± standard deviation. Increases are post-cycle-two minus baseline; declines are baseline minus post-cycle-two. CRP, C-reactive protein; GPx, glutathione peroxidase; GSH, reduced glutathione; IL-6, interleukin-6; MDA, malondialdehyde; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α. P values are unadjusted. CRP change is descriptive because baseline CRP differed between groups.

Table 4: Baseline Routine Laboratory and Inflammatory Indices

Variable	Total (N=150)	No composite classification (n=108)	Composite classification (n=42)	P value
Hemoglobin, g/dL	12.1 ± 1.2	12.1 ± 1.1	12.2 ± 1.4	0.745
Creatinine, mg/dL	0.83 ± 0.17	0.82 ± 0.15	0.85 ± 0.19	0.324
ALT, U/L	26.8 ± 13.9	26.6 ± 13.1	27.1 ± 16.2	0.861
Albumin, g/dL	4.06 ± 0.36	4.05 ± 0.35	4.08 ± 0.38	0.686
Neutrophil-to-lymphocyte ratio	2.40 ± 1.12	2.38 ± 1.11	2.44 ± 1.15	0.793
Platelet-to-lymphocyte ratio	156.9 ± 59.9	156.6 ± 65.0	157.9 ± 44.8	0.889
Systemic immune-inflammation index	633.7 ± 309.3	633.5 ± 332.4	634.4 ± 243.3	0.984

Data are mean ± standard deviation. ALT, alanine aminotransferase. P values are unadjusted.

Table 5: Composite Classification and Early Clinical Consequences

Variable	Total (N=150)	No composite classification (n=108)	Composite classification (n=42)	P value
Classification category				NA
None	108 (72.0)	108 (100.0)	0 (0.0)	
Biomarker-defined myocardial injury	10 (6.7)	0 (0.0)	10 (23.8)	
Clinical CTRCD	32 (21.3)	0 (0.0)	32 (76.2)	
Cardiac symptoms				<0.001
No	134 (89.3)	106 (98.1)	28 (66.7)	
Yes	16 (10.7)	2 (1.9)	14 (33.3)	
Cardiology referral				<0.001
No	117 (78.0)	101 (93.5)	16 (38.1)	
Yes	33 (22.0)	7 (6.5)	26 (61.9)	
Chemotherapy delay due to cardiac concern				<0.001
No	132 (88.0)	102 (94.4)	30 (71.4)	
Yes	18 (12.0)	6 (5.6)	12 (28.6)	

Data are n (%). CTRCD, cancer therapyrelated cardiac dysfunction. Cardiac symptoms, cardiology referral, and chemotherapy delay were consequences and were not used alone to define clinical CTRCD. No P value was calculated for classification category because that category defines group membership.

years, diabetes, and planned totalcourse doxorubicinequivalent dose ≥300 mg/m² were not statistically significant (Table **6**). Postcycletwo biomarkers were not included in these analyses.

Standardized Early Change-Score Differences

Figure **1** displays Hedges g estimates for adverse change scores. All estimates favored larger adverse change in the composite group, with the largest standardized differences observed for NT-proBNP increase, LVEF decline, MDA increase, CK-MB increase, troponin increase, TNF-α increase, and GSH decline. These estimates are descriptive and unadjusted; the troponin and NT-proBNP estimates are affected by incorporation into the biomarker-defined classification.

DISCUSSION

This prospective clinical toxicology study characterized an early, concurrent multi-pathway phenotype after two anthracycline cycles. Participants meeting the composite cardiac injury/CTRCD classification had greater myocardial-injury and wall-stress biomarker changes, larger LVEF decline, more oxidative damage, greater antioxidant depletion, and stronger inflammatory activation. Among baseline factors, hypertension and LVEF <60% were associated with the composite classification. The revised analysis intentionally does not describe post-cycle-two biomarkers as predictors because biomarker sampling and cardiac classification occurred during the same assessment window, and troponin or NT-proBNP contributed directly to classification in 10 participants.

This distinction addresses both temporal ambiguity and incorporation bias. A true prediction analysis requires a candidate marker measured before an independently defined later outcome. In the present study, the post-cycle-two biomarkers can support concurrent early detection and biological characterization, but the data cannot establish that these measurements predict a subsequent event. Consequently, the previous biomarker odds ratios, ROC analyses, and perfect combined-score discrimination were removed rather than interpreted as clinical performance.

Troponin and NT-proBNP remain clinically relevant because they are routinely available and are included

Table 6: Univariable Associations of Baseline Clinical Factors with the Composite Classification

Baseline factor	Odds ratio	95% CI	P value
Age ≥55 years	0.70	0.31–1.56	0.427
Hypertension	2.87	1.36–6.21	0.006
Diabetes mellitus	2.21	0.79–6.21	0.149
Planned totalcourse doxorubicinequivalent dose ≥300 mg/m²	1.85	0.85–4.04	0.142
Baseline LVEF <60%	2.53	1.17–5.50	0.021

CI, confidence interval; LVEF, left ventricular ejection fraction. The outcome was the composite early cardiac injury/CTRCD classification. Post-cycle-two biomarkers were excluded because they were measured concurrently and, for troponin and NT-proBNP, partly defined the outcome.

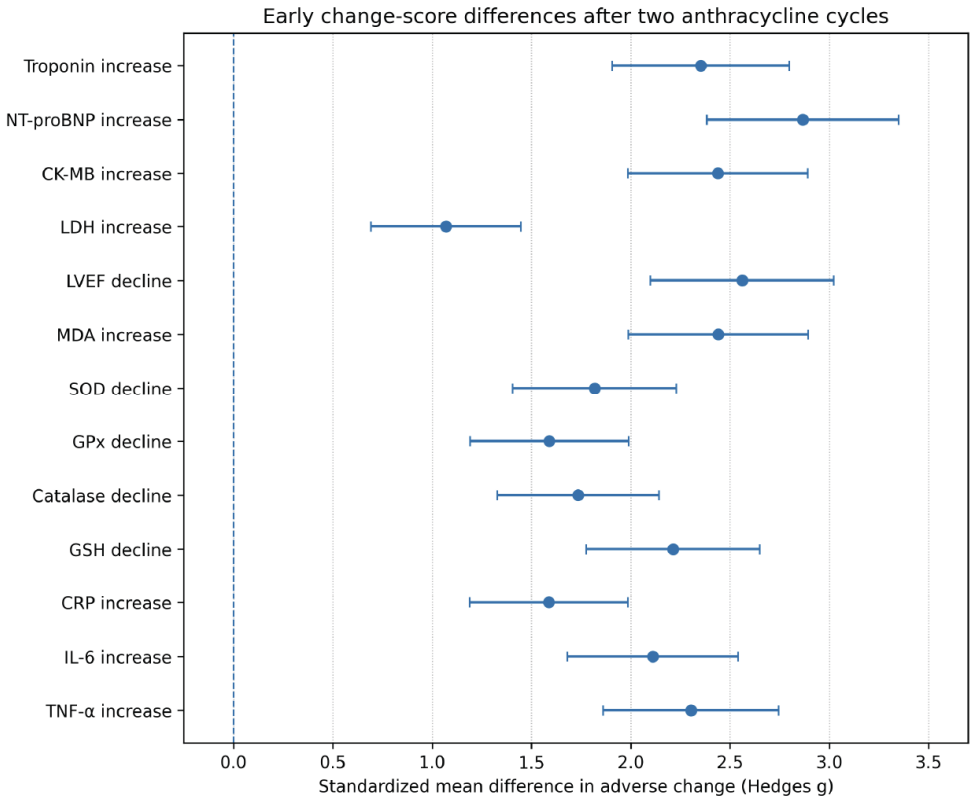

Figure 1: Standardized betweengroup differences in early adverse change scores after two anthracycline cycles. Hedges g values and 95% CIs compare participants with the composite early cardiac injury/CTRCD classification (n=42) with those without the classification (n=108). Positive values indicate a larger adverse change in the composite group; LVEF and antioxidant outcomes are plotted as the magnitude of decline. Estimates were derived from the summary means and standard deviations in Tables **2** and **3** and are descriptive and unadjusted. Troponin and NTproBNP contributed to biomarkerdefined myocardial injury in 10 participants, so their effect sizes do not represent independent diagnostic or predictive performance. The summary source data and standardized effectsize estimates underlying this figure are provided in Supplementary Table **S2**.

in contemporary cardio-oncology surveillance frameworks [4,8-12,21-24]. Their baseline concentrations were broadly similar between groups, whereas their post-cycle-two changes were larger in the composite group. However, the magnitude of these differences is partly expected by construction for the 10 biomarker-defined cases. Future studies should measure these biomarkers at a prespecified earlier time point and evaluate later CTRCD defined independently by symptoms, LVEF, and preferably global longitudinal strain.

The oxidative-stress findings are biologically coherent with established anthracycline mechanisms. Doxorubicin can generate reactive oxygen species, impair mitochondrial energetics, promote lipid

peroxidation, and activate multiple cell-death pathways [13,25-27]. The larger MDA increase and greater declines in SOD, GPx, catalase, and GSH therefore support a state of treatment-emergent oxidative imbalance. These markers are nevertheless mainly research assays; variability in specimen handling, analytical methods, and reference ranges limits direct translation to routine care.

Inflammatory activation was also more pronounced in the composite group, but interpretation requires caution. Baseline CRP was already higher in this group, and the study did not estimate a baseline-adjusted CRP effect. The post-cycle-two CRP, IL-6, and TNF-α changes therefore indicate concurrent inflammatory activation rather than independent cardiac specificity. Oxidative stress and inflammation are mechanistically interconnected, and both can also be influenced by cancer burden, infection, obesity, and other comorbid conditions [15,28,29].

Hypertension and lowernormal baseline LVEF may identify patients with reduced cardiovascular reserve. Their associations are consistent with cardiooncology risk frameworks that emphasize preexisting cardiovascular disease, hypertension, age, prior cardiotoxic therapy, and baseline imaging or biomarker abnormalities [4,30,31]. The planned totalcourse doxorubicinequivalent dose was not statistically significant in this early assessment. Because this variable represented the intended full regimen rather than the dose actually administered by cycle two, the analysis does not test the shortterm effect of delivered cumulative exposure. Dose received remains particularly important for later toxicity risk [3,4,32].

A clinically plausible surveillance pathway would combine baseline cardiovascular risk assessment with scheduled echocardiography and clinically available troponin and NT-proBNP measurements. A marked same-window biomarker rise, new symptoms, or an LVEF decline could prompt repeat imaging, review of reversible causes, and cardio-oncology referral. In contrast, MDA, SOD, GPx, catalase, GSH, IL-6, and TNF-α should remain investigational until assays, thresholds, incremental value, and clinical utility are validated. This study does not support changing or withholding cancer therapy on the basis of any single biomarker.

The study has several strengths: prospective data collection; paired baseline and post-cycle-two measurements; integration of cardiac, oxidative, antioxidant, and inflammatory pathways; and documentation of clinically relevant consequences. The revised presentation also separates concurrent detection from prediction and limits regression to baseline factors.

Several limitations remain. The study was conducted in one national setting and had a moderate sample size. Followup ended after cycle two, precluding assessment of later CTRCD. Global longitudinal strain was not available. The composite classification included 10 cases defined by troponin and/or NTproBNP, creating incorporation bias for descriptive comparisons of those markers. Multiple biomarker comparisons were exploratory and unadjusted, and CRP analyses were not adjusted for the baseline difference. The regression analyses were univariable and residual confounding is likely. The anthracycline dose actually administered through cycle two was not separately modeled. The TNFα assay's lower measuring limit was close to several baseline concentrations, which may have reduced precision near the lower end of the range. Although body mass index and major baseline cardiac exclusions were recorded, smoking status, previous chest radiotherapy, detailed prior cardiovascular disease, and concurrent cardioprotective medication use were not systematically captured. These factors should be prespecified in future multicenter studies.

Overall, the results identify a coherent early phenotype of anthracycline-associated cardiac injury and dysfunction, but they should be regarded as hypothesis-generating. Temporal prediction and clinical decision thresholds require a design with clearly separated biomarker and outcome time points, an outcome defined independently of the candidate markers, adequate event numbers, prespecified modeling, and external validation.

CONCLUSION

After two anthracycline cycles, participants with a composite classification of biomarker-defined myocardial injury or clinical CTRCD showed a concurrent profile of myocardial injury, wall stress, LVEF decline, oxidative damage, antioxidant depletion, and inflammation. Hypertension and baseline LVEF <60% were associated with this classification in univariable analyses. Because biomarkers and cardiac status were assessed during the same post-cycle-two window, the findings support concurrent early detection and biological characterization rather than temporal prediction. Larger multicenter studies with independent outcome definitions, earlier biomarker sampling, global

longitudinal strain, comprehensive confounder measurement, and longer follow-up are required before biomarker-guided clinical decisions can be recommended.

CONSENT FOR PUBLICATION

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COMPETING INTERESTS

The author declares no competing interests.

DATA AVAILABILITY

De-identified participant-level data and the statistical analysis file are available from the corresponding author on reasonable request, subject to ethics approval and applicable data-protection requirements.

AUTHOR CONTRIBUTIONS

A.M.M. conceived the study, developed the protocol, coordinated data collection and laboratory assessment, analyzed and interpreted the data, drafted the manuscript, critically revised the work, approved the final version, and accepts accountability for the integrity of the study.

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SUPPLEMENTARY MATERIAL

Supplementary Methods and Supplementary Tables **S1** and **S2** are available with the online article.

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