

BASELINE CLINICAL, ECHOCARDIOGRAPHIC, AND BIOMARKER PREDICTORS OF EARLY NT-PROBNP AND GALECTIN-3 DETERIORATION DURING ANTHRACYCLINE CHEMOTHERAPY: A PLACEBO-ARM SECONDARY ANALYSIS

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ABSTRACT

Background: Anthracycline cardiotoxicity may begin with subclinical biomarker deterioration before overt left ventricular dysfunction develops. Whether routine baseline variables can identify patients who subsequently develop early biomarker worsening remains uncertain. This exploratory secondary analysis evaluated whether baseline clinical, echocardiographic, and biomarker characteristics were associated with 4-month changes in NT-proBNP and galectin-3 during anthracycline-based chemotherapy.

Methods: This analysis included placebo-treated participants from a prospective, randomized, double-blind, placebo-controlled trial evaluating cardioprotection during anthracycline therapy. Only the placebo arm was analyzed to assess the natural course of biomarker changes without active cardioprotective treatment. The final cohort included 45 patients with preserved baseline cardiac function and paired baseline and 4-month NT-proBNP and galectin-3 measurements. Candidate baseline predictors included age, sex, body surface area, left ventricular ejection fraction, E/A ratio, NT-proBNP, and galectin-3. Associations with absolute biomarker changes were assessed using Spearman correlation and exploratory simple linear regression.

Results: NT-proBNP increased from 47.96 ± 45.98 pg/mL to 73.62 ± 55.24 pg/mL, with a mean change of $+25.67 \pm 38.43$ pg/mL. Galectin-3 increased from 379.1 ± 158.8 pg/mL to 438.7 ± 186.2 pg/mL, with a mean change of $+59.56 \pm 117.1$ pg/mL. Any increase occurred in 77.8% of patients for NT-proBNP and 68.9% for galectin-3. No predefined baseline clinical, echocardiographic, or biomarker variable was significantly associated with Δ NT-proBNP or Δ Galectin-3.

Conclusion: Early NT-proBNP and galectin-3 deterioration was common during anthracycline chemotherapy despite preserved baseline cardiac function. Routine baseline variables showed limited ability to identify patients who later developed biomarker worsening, supporting serial biomarker surveillance and dynamic risk assessment.

KEYWORDS: anthracycline cardiotoxicity; NT-proBNP; galectin-3; cardio-oncology; biomarker surveillance.

INTRODUCTION

Anthracyclines remain essential in the treatment of breast cancer, lymphoma, leukemia, and other malignancies, but their clinical utility is constrained by cardiovascular toxicity that can impair quality of life, compromise cancer treatment delivery, and worsen survival (1–3). Contemporary evidence supports a model in which anthracycline cardiotoxicity begins as subclinical myocardial injury and progresses over time to measurable ventricular dysfunction and, in some patients, symptomatic heart failure (1,4,5). This temporal sequence has major clinical implications because recovery is more likely when toxicity is recognized early, before overt reduction in left ventricular ejection fraction (LVEF) becomes established (1,4).

Surveillance Strategies

Current cardio-oncology recommendations therefore emphasize baseline risk assessment and serial cardiovascular surveillance during potentially cardiotoxic therapy (2,6,7). In practice, surveillance typically integrates clinical risk

profiling, echocardiography, and circulating biomarkers, although guideline harmonization remains imperfect because the supporting evidence base is still limited and heterogeneous (2,7,8). Echocardiography is central because it is widely available, noninvasive, and serially repeatable, but conventional LVEF has limited sensitivity for early injury and often changes only after more subtle myocardial damage has already occurred (5,9,10).

This limitation is evident in prospective anthracycline cohorts. In the AIC registry, cardiotoxicity occurred in 11.0% of patients over 2 years, and 3-month biomarker and strain changes, rather than baseline measures alone, were independently associated with subsequent cardiotoxicity (4). In a 72-patient prospective study, baseline LVEF showed some predictive value for later cardiotoxicity, but NT-proBNP did not, and post-anthracycline imaging parameters such as LV end-systolic volume and global longitudinal strain were better early detectors (11). More advanced imaging may improve discrimination, but even recent studies show that pre-chemotherapy echocardiographic parameters do not uniformly stratify risk, reinforcing that baseline LVEF alone is often insufficient for identifying patients who will later manifest myocardial injury (12).

Biomarker Rationale

Circulating biomarkers provide a complementary strategy because they can reflect biological injury before structural dysfunction is fully expressed on imaging (8). NT-proBNP is primarily interpreted as a marker of myocardial wall stress, and anthracycline studies show that natriuretic peptide concentrations often rise during treatment even when overt systolic dysfunction is not yet apparent (1,11). In one prospective cohort, baseline NT-proBNP was the strongest predictor of subsequent LVEF change, while post-therapy NT-proBNP retained similar predictive value (13). In contrast, other studies have found that baseline or early NT-proBNP measurements do not predict later cardiotoxicity, underscoring that the literature is inconsistent rather than uniformly positive (1,11).

Galectin-3 is biologically attractive because it is linked to inflammation, extracellular matrix remodeling, and fibrosis, processes implicated in anthracycline-related myocardial injury (14). However, galectin-3 has been far less studied than troponins and natriuretic peptides in cardio-oncology surveillance (8). The available evidence is mixed. Interim galectin-3 changes have correlated with 6-month LVEF change and with GLS change in a small prospective anthracycline cohort (13). By contrast, broader summaries note that pretreatment galectin-3 has not consistently predicted cardiovascular outcomes, and some studies found no association with later LVEF decline or long-term dysfunction (1,15). This discrepancy supports examining galectin-3 not only as a static baseline value but also as a serial marker of evolving myocardial stress and remodeling.

Baseline Predictors

The broader literature on baseline predictors of anthracycline cardiotoxicity is similarly heterogeneous. Large registry data validate multivariable baseline risk stratification frameworks such as HFA-ICOS, with higher baseline risk categories associated with substantially greater rates of clinically relevant anthracycline cardiovascular toxicity and mortality (6). Yet these tools aggregate multiple comorbid, treatment, and demographic features and do not answer a narrower question that is highly relevant to routine practice: whether simple pretreatment variables available in most oncology settings can identify patients who will later show biomarker deterioration despite initially reassuring cardiac evaluation.

Evidence for individual baseline factors is mixed. Age was not a significant predictor in one prospective study of anthracycline and trastuzumab exposure (11), but older age and conventional cardiovascular risk factors were associated with subclinical toxicity in a lymphoma cohort (16). Age and sex were not associated with cardiotoxicity in one pediatric anthracycline study (17), and body surface area is rarely isolated as a predictor in recent biomarker-focused anthracycline studies despite its routine clinical availability. Baseline LVEF has shown predictive value in some cohorts (11), but not all baseline imaging approaches stratify risk effectively (12). Baseline natriuretic peptides tend to associate with later events in some reports, but results remain sparse (1). Most importantly, few recent studies specifically test whether baseline clinical, echocardiographic, and biomarker characteristics predict subsequent increases in NT-proBNP or galectin-3, rather than later CTRCD, GLS decline, or overt LVEF reduction.

A placebo-arm secondary analysis offers a useful natural-history cohort for addressing this question because it avoids confounding by active cardioprotective therapy and isolates biomarker trajectories during anthracycline exposure under standard monitoring. Clarifying whether routine baseline characteristics identify patients prone to rising NT-proBNP and galectin-3 could refine pretreatment risk stratification and inform whether serial biomarker surveillance remains necessary even among patients who appear low risk at baseline. Therefore, this placebo-arm secondary analysis aimed to determine whether baseline clinical characteristics (age, sex, and body surface area), baseline echocardiographic parameters (left ventricular ejection fraction and E/A ratio), and baseline biomarkers (NT-proBNP and galectin-3) are associated with subsequent 4-month changes in NT-proBNP and galectin-3 during anthracycline-based chemotherapy.

MATERIALS AND METHODS

Study Design and Rationale

This study was designed as an exploratory secondary analysis of the placebo arm of a prospective, randomized, double-blind, placebo-controlled trial evaluating dapagliflozin for the prevention of anthracycline-associated cardiotoxicity (ClinicalTrials.gov Identifier: NCT06888505). The present analysis was not intended to assess treatment efficacy or to compare randomized treatment groups. Instead, it focused exclusively on placebo-treated participants in order to evaluate the natural course of early biomarker changes during anthracycline chemotherapy in the absence of active cardioprotective therapy.

The study was structured as a prediction and risk-stratification analysis. The primary objective was to determine whether routinely available baseline clinical, echocardiographic, and biomarker characteristics were associated with subsequent deterioration in cardiac biomarkers over a 4-month period of anthracycline-based chemotherapy. The analysis specifically focused on early changes in NT-proBNP and galectin-3, representing complementary biomarkers of myocardial stress and profibrotic or inflammatory activation.

Study Setting and Participants

Participants were recruited from Omed Oncology Teaching Hospital, formerly Azadi Oncology Center, in Duhok, Kurdistan Region, Iraq. The parent trial enrolled adult patients with newly diagnosed malignancy who were scheduled to receive anthracycline-based chemotherapy and had preserved baseline cardiac function. For the present secondary analysis, only participants randomized to placebo in the parent trial were included.

A total of 47 participants were initially allocated to the placebo arm. Two participants withdrew before completing follow-up assessments and were therefore excluded from the present complete-case analysis. The final analytical cohort consisted of 45 placebo-treated patients who had complete baseline and 4-month measurements of NT-proBNP and galectin-3.

Eligible participants in the parent trial were adults aged 18 years or older, had no previous exposure to anthracycline chemotherapy, and had preserved left ventricular systolic function at baseline. Patients with known heart failure, established cardiomyopathy, significant valvular heart disease, uncontrolled hypertension, severe renal dysfunction, recent sodium–glucose cotransporter 2 inhibitor use, or prior chest radiotherapy were excluded from the parent trial. These criteria were applied before randomization in the original study and are described here to clarify the clinical characteristics of the analyzed placebo cohort.

Candidate Baseline Predictors

Candidate baseline predictors were selected a priori based on their clinical relevance and their frequent use in cardio-oncology risk assessment. The selected variables were intentionally limited to routinely obtainable characteristics in order to preserve the practical risk-stratification focus of the analysis and to reduce model complexity given the exploratory sample size.

Clinical candidate predictors included age, sex, and body surface area. Baseline echocardiographic candidate predictors included left ventricular ejection fraction and transmitral E/A ratio. Baseline biomarker candidate predictors included NT-proBNP and galectin-3. These variables were evaluated as potential predictors of subsequent biomarker deterioration during chemotherapy. Baseline echocardiographic variables were not analyzed as treatment outcomes in this manuscript; rather, they were included only as candidate predictors of later biomarker change.

Biomarker Assessment

Venous blood samples were obtained at baseline before initiation of anthracycline chemotherapy and again at the 4-month follow-up visit. Samples were processed according to standardized local laboratory procedures. Serum aliquots were stored at -60°C until analysis. Biomarker measurements were performed in batches to minimize inter-assay variability and reduce analytical drift between baseline and follow-up samples.

NT-proBNP was measured using the Roche Cobas e601 electrochemiluminescence immunoassay platform. Galectin-3 was measured using a commercially available enzyme-linked immunosorbent assay kit according to the manufacturer's instructions. Laboratory personnel performed analyses using standardized procedures and quality-control measures appropriate for each assay. The present analysis used absolute baseline concentrations and absolute 4-month changes in each biomarker.

Echocardiographic Assessment

Baseline transthoracic echocardiography was performed before the initiation of chemotherapy. Echocardiographic assessment included measurement of left ventricular ejection fraction and transmitral Doppler indices. Left ventricular

ejection fraction was used as a marker of baseline systolic function, while E/A ratio was used as a routinely available Doppler marker of diastolic filling pattern.

For the present secondary analysis, echocardiographic variables were used only as baseline candidate predictors. No comparison of serial echocardiographic treatment effects was performed. This approach was selected to maintain the specific aim of the manuscript: determining whether baseline cardiac structure or function, within an apparently preserved range, identifies patients at increased risk of early biomarker deterioration during anthracycline chemotherapy.

Outcomes

The primary outcomes of this analysis were the absolute 4-month changes in NT-proBNP and galectin-3. For each biomarker, change was calculated as the follow-up value minus the baseline value. Positive values therefore indicated biomarker increase over time, whereas negative values indicated biomarker reduction.

Secondary descriptive outcomes included the proportion of patients showing any increase in NT-proBNP or galectin-3 from baseline to 4 months. Additional descriptive thresholds were used to classify patients according to relative biomarker increase of at least 25%, at least 50%, and at least 100% from baseline. These thresholds were selected for descriptive and exploratory purposes only. They should not be interpreted as validated diagnostic cut-offs for anthracycline cardiotoxicity, myocardial injury, heart failure, or clinically meaningful cardiotoxic events.

Statistical Analysis

Continuous variables were summarized as mean $\pm$ standard deviation for approximately normally distributed data and as median with interquartile range for non-normally distributed data. Categorical variables were summarized as frequency and percentage. The distribution of continuous variables was assessed using the Shapiro–Wilk test, supported by visual inspection where appropriate.

The primary analytical approach evaluated associations between baseline candidate predictors and subsequent biomarker deterioration using Spearman rank correlation. Spearman correlation was selected because biomarker concentrations and biomarker changes may show non-normal distributions, skewness, and outlying values in oncology populations. Correlation analyses were performed between each predefined baseline predictor and each biomarker change outcome. Specifically, age, sex, body surface area, baseline left ventricular ejection fraction, baseline E/A ratio, baseline NT-proBNP, and baseline galectin-3 were evaluated in relation to Δ NT-proBNP and Δ galectin-3.

Exploratory simple linear regression analyses were also performed to estimate the direction and magnitude of associations between selected baseline predictors and absolute biomarker changes. These models were not intended to generate definitive prediction equations or externally validated risk models. Rather, they were used to complement correlation analyses by describing the approximate effect direction and size of candidate predictor–outcome relationships.

No formal adjustment for multiple comparisons was performed. Therefore, statistically significant findings should be interpreted in the context of exploratory testing and the potential for type I error. A two-sided P value <0.05 was considered statistically significant. All statistical analyses were performed using JASP version 0.96 (JASP Team, University of Amsterdam, Amsterdam, the Netherlands).

Ethical Considerations

The parent randomized clinical trial received ethical approval from the Ethics Committee of the College of Medicine, Hawler Medical University, and from the Duhok General Directorate of Health Ethics Committee. All participants provided written informed consent before enrollment. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The present secondary analysis used data collected within the approved parent trial and was limited to placebo-treated participants with complete baseline and follow-up biomarker data.

RESULTS

Study Population

Of the 47 placebo-treated participants in the parent randomized clinical trial, two withdrew before completing follow-up assessments. The final analysis included 45 patients with complete baseline and 4-month measurements of NT-proBNP and galectin-3. No missing data were present for the analyzed clinical, echocardiographic, or biomarker variables.

The baseline characteristics of the study population are summarized in Table 1. The cohort was predominantly female, with 39 women (86.7%) and 6 men (13.3%). The mean age was 46.42 ± 12.04 years, and the mean body surface area was 1.70 ± 0.13 m². Baseline systolic function was preserved, with a mean LVEF of $60.78 \pm 2.60\%$. The mean baseline

E/A ratio was 0.98 ± 0.09 . Baseline NT-proBNP and galectin-3 showed non-normal distributions, with median values of 37.00 pg/mL and 347.9 pg/mL, respectively.

Table 1. Baseline Clinical, Echocardiographic, and Biomarker Characteristics

Variable	Value
Number of patients	45
Age, years	46.42 ± 12.04
Female sex, n (%)	39 (86.7%)
Male sex, n (%)	6 (13.3%)
Body surface area, m ²	1.70 ± 0.13
Baseline LVEF, %	60.78 ± 2.60
Baseline E/A ratio	0.98 ± 0.09
Baseline NT-proBNP, pg/mL	37.00 (IQR 27.00)
Baseline galectin-3, pg/mL	347.9 (IQR 136.8)

Values are presented as mean $\pm$ SD or median (IQR), as appropriate. Abbreviations: IQR, interquartile range; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SD, standard deviation.

Four-Month Changes in NT-proBNP and Galectin-3

Baseline, follow-up, and absolute biomarker changes are presented in Table 2. Over 4 months of anthracycline chemotherapy, both biomarkers demonstrated an overall increase, although individual responses varied substantially. NT-proBNP increased from a mean baseline value of 47.96 ± 45.98 pg/mL to 73.62 ± 55.24 pg/mL at 4 months, corresponding to a mean absolute change of $+25.67 \pm 38.43$ pg/mL. Galectin-3 increased from 379.1 ± 158.8 pg/mL at baseline to 438.7 ± 186.2 pg/mL at 4 months, with a mean absolute change of $+59.56 \pm 117.1$ pg/mL.

The median absolute increase was $+16.00$ pg/mL for NT-proBNP and $+33.17$ pg/mL for galectin-3. The median relative increase was 36.00% for NT-proBNP and 10.00% for galectin-3, indicating that relative biomarker worsening was more pronounced for NT-proBNP than for galectin-3.

Table 2. Baseline, Follow-Up, and Absolute Biomarker Changes

Biomarker	Baseline	4 months	Absolute change	Percent change
NT-proBNP, pg/mL, mean $\pm$ SD	47.96 ± 45.98	73.62 ± 55.24	$+25.67 \pm 38.43$	$+95.33 \pm 133.3$
NT-proBNP, pg/mL, median (IQR)	37.00 (27.00)	64.00 (54.00)	$+16.00$ (41.00)	$+36.00$ (136.0)
Galectin-3, pg/mL, mean $\pm$ SD	379.1 ± 158.8	438.7 ± 186.2	$+59.56 \pm 117.1$	$+22.76 \pm 49.35$
Galectin-3, pg/mL, median (IQR)	347.9 (136.8)	398.0 (188.0)	$+33.17$ (136.0)	$+10.00$ (41.00)

Absolute change was calculated as follow-up value minus baseline value. Percent change was calculated as $[(\text{follow-up} - \text{baseline}) / \text{baseline}] \times 100$.

Abbreviations: IQR, interquartile range; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SD, standard deviation.

Descriptive Biomarker Deterioration Thresholds

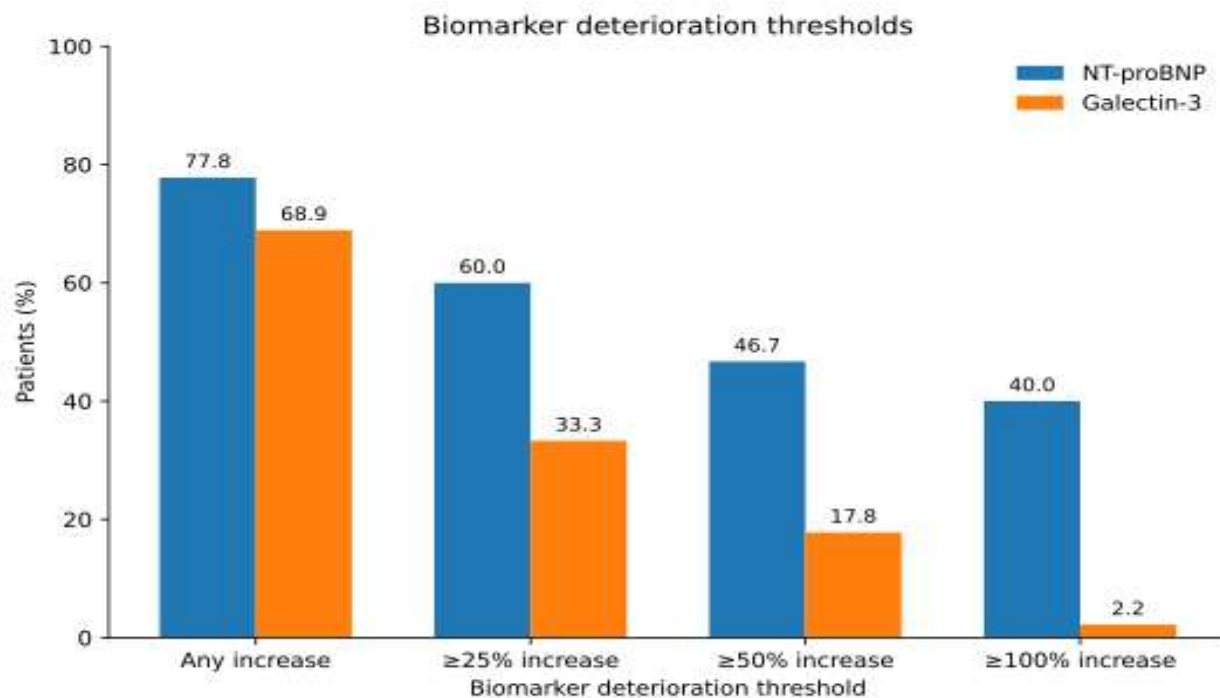
The frequency of biomarker deterioration according to descriptive relative-change thresholds is summarized in Table 3. Any increase in NT-proBNP was observed in 35 patients (77.8%), while any increase in galectin-3 was observed in 31 patients (68.9%). Relative increases of $\geq 25\%$, $\geq 50\%$, and $\geq 100\%$ were more frequent for NT-proBNP than for galectin-3. A $\geq 100\%$ increase occurred in 18 patients (40.0%) for NT-proBNP but in only one patient (2.2%) for galectin-3. Overall, biomarker deterioration was more frequent and more pronounced for NT-proBNP than for galectin-3, particularly at the higher relative-change thresholds (Figure 1).

These thresholds were used for descriptive purposes only and should not be interpreted as validated diagnostic cut-offs for cardiotoxicity, myocardial injury, or heart failure.

Table 3. Frequency of Biomarker Deterioration According to Descriptive Relative-Change Thresholds

Deterioration threshold	NT-proBNP, n (%)	Galectin-3, n (%)
Any increase	35 (77.8%)	31 (68.9%)
≥25% increase	27 (60.0%)	15 (33.3%)
≥50% increase	21 (46.7%)	8 (17.8%)
≥100% increase	18 (40.0%)	1 (2.2%)

Thresholds were used for descriptive purposes only and should not be interpreted as validated diagnostic cut-offs for cardiotoxicity, myocardial injury, or heart failure. Abbreviation: NT-proBNP, N-terminal pro-B-type natriuretic peptide.

**Figure 1.** Frequency of NT-proBNP and galectin-3 deterioration during anthracycline chemotherapy.

Bars show the proportion of placebo-treated patients who experienced any increase or relative increases of ≥25%, ≥50%, and ≥100% from baseline to 4 months. These thresholds were used descriptively and should not be interpreted as validated diagnostic cut-offs.

Associations Between Baseline Predictors and Biomarker Changes

Spearman correlation analyses were performed to evaluate whether baseline clinical, echocardiographic, or biomarker variables were associated with subsequent absolute biomarker changes. The correlation results are summarized in Table 4. None of the predefined baseline predictors showed a statistically significant association with Δ NT-proBNP or Δ Galectin-3.

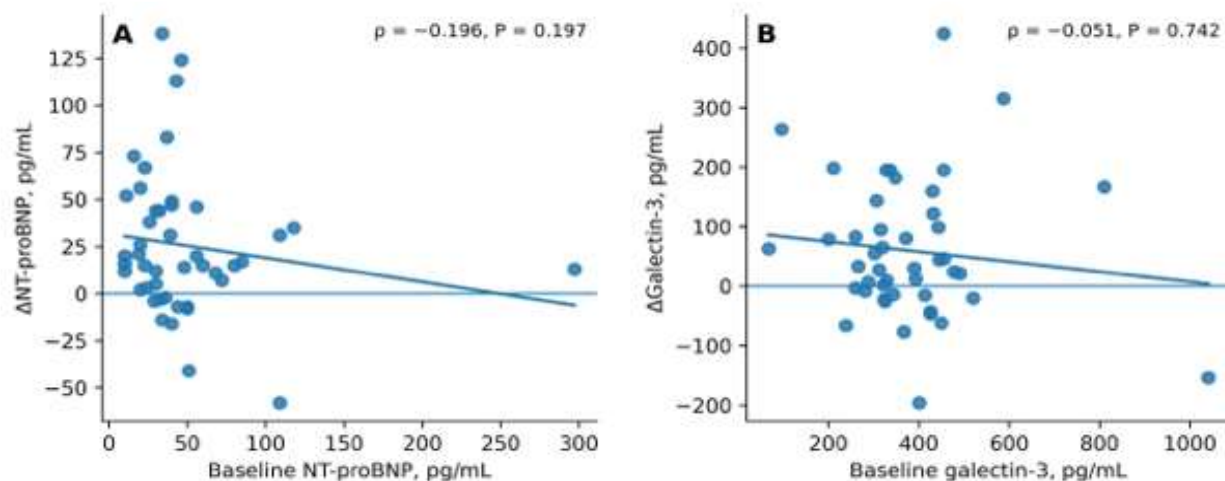
For Δ NT-proBNP, weak positive correlations were observed with age, body surface area, and baseline LVEF; however, none reached statistical significance. Baseline NT-proBNP showed a weak inverse association with Δ NT-proBNP, but this association was also not statistically significant. For Δ Galectin-3, all correlations were weak and non-significant. The strongest numerical association was a weak inverse correlation between baseline LVEF and Δ Galectin-3.

Baseline NT-proBNP was not significantly associated with Δ NT-proBNP, and baseline galectin-3 was not significantly associated with Δ Galectin-3. The absence of a clear relationship between baseline biomarker concentrations and subsequent biomarker changes is illustrated in Figure 2.

Table 4. Spearman Correlations Between Baseline Predictors and Absolute Biomarker Changes

Baseline predictor	Δ NT-proBNP Spearman ρ	P value	Δ Galectin-3 Spearman ρ	P value
Age	0.180	0.236	-0.087	0.571
Sex	-0.151	0.322	-0.005	0.974
Body surface area	0.187	0.220	-0.021	0.894
Baseline LVEF	0.197	0.194	-0.210	0.165
Baseline E/A ratio	0.011	0.944	0.108	0.479
Baseline NT-proBNP	-0.196	0.197	0.054	0.725
Baseline galectin-3	0.165	0.278	-0.051	0.742

Sex was included as a binary coded variable; therefore, the direction of the correlation coefficient depends on coding and should be interpreted cautiously. Abbreviations: LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

**Figure 2.** Relationship between baseline biomarker concentrations and subsequent biomarker changes.

Scatter plots show the association between baseline NT-proBNP and Δ NT-proBNP (A), and baseline galectin-3 and Δ Galectin-3 (B). Spearman correlation coefficients and P values are shown within each panel. No statistically significant association was observed between baseline biomarker concentrations and subsequent absolute biomarker deterioration. The fitted line is shown for visual orientation only.

Simple Linear Regression

Exploratory simple linear regression analyses were consistent with the Spearman correlation findings. The regression results are summarized in Table 5. None of the baseline variables significantly predicted absolute change in NT-proBNP or galectin-3.

For Δ NT-proBNP, age showed a non-significant positive association, with an estimated increase of 0.809 pg/mL in Δ NT-proBNP per one-year increase in age. Baseline LVEF also showed a non-significant positive association with Δ NT-proBNP. However, both associations remained above the predefined statistical significance threshold.

For Δ Galectin-3, baseline LVEF showed the strongest numerical association, with a non-significant inverse relationship. No other baseline clinical, echocardiographic, or biomarker variable demonstrated evidence of a statistically significant linear association with Δ Galectin-3.

Table 5. Simple Linear Regression for Absolute Biomarker Changes

Outcome	Predictor	β coefficient	95% CI	P value
Δ NT-proBNP, pg/mL	Age	0.809	-0.140 to 1.759	0.093
Δ NT-proBNP, pg/mL	Body surface area	40.33	-50.59 to 131.2	0.376
Δ NT-proBNP, pg/mL	Baseline LVEF	3.683	-0.716 to 8.082	0.099

ΔNT-proBNP, pg/mL	Baseline E/A ratio	53.32	−70.81 to 177.4	0.391
ΔNT-proBNP, pg/mL	Baseline NT-proBNP	−0.128	−0.382 to 0.126	0.317
ΔNT-proBNP, pg/mL	Baseline galectin-3	0.003	−0.071 to 0.078	0.934
ΔGalectin-3, pg/mL	Age	−1.258	−4.225 to 1.709	0.397
ΔGalectin-3, pg/mL	Body surface area	−103.2	−381.1 to 174.7	0.458
ΔGalectin-3, pg/mL	Baseline LVEF	−10.77	−24.22 to 2.673	0.113
ΔGalectin-3, pg/mL	Baseline E/A ratio	156.0	−222.6 to 534.6	0.411
ΔGalectin-3, pg/mL	Baseline NT-proBNP	0.230	−0.550 to 1.010	0.555
ΔGalectin-3, pg/mL	Baseline galectin-3	−0.084	−0.310 to 0.141	0.455

β coefficients are unstandardized.

Abbreviations: CI, confidence interval; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Summary of Main Findings

In this exploratory placebo-arm secondary analysis, early increases in NT-proBNP and galectin-3 were common during anthracycline chemotherapy. NT-proBNP showed more frequent and larger relative increases than galectin-3, including a ≥100% increase in 40.0% of patients. However, routine baseline clinical variables, baseline echocardiographic parameters, and baseline biomarker concentrations did not significantly predict subsequent absolute deterioration in either NT-proBNP or galectin-3 over 4 months. These findings suggest that, within this preserved baseline cardiac-function cohort, routine baseline assessment alone had limited ability to identify patients who subsequently developed early biomarker worsening during anthracycline therapy.

DISCUSSION

In this exploratory secondary analysis of placebo-treated patients undergoing anthracycline-based chemotherapy, early deterioration of NT-proBNP and galectin-3 over 4 months was common despite preserved baseline cardiac function. NT-proBNP increased in 77.8% of participants and galectin-3 in 68.9%, with larger and more frequent relative increases observed for NT-proBNP. In contrast, routine baseline clinical characteristics, conventional baseline echocardiographic measures, and baseline biomarker concentrations did not significantly identify patients who subsequently developed greater absolute biomarker deterioration. These findings are clinically relevant because they suggest that apparently reassuring baseline assessment may not be sufficient to exclude early biological evidence of cardiovascular stress during anthracycline exposure. However, they should be interpreted cautiously because the outcomes were biomarker changes rather than adjudicated cancer therapy-related cardiac dysfunction (CTRCD), symptomatic heart failure, or longitudinal imaging deterioration.

This pattern is biologically plausible. NT-proBNP primarily reflects myocardial wall stress and subclinical hemodynamic strain, and natriuretic peptide elevations may occur during anthracycline treatment before overt reductions in left ventricular ejection fraction (LVEF) become apparent (11,18). In a prospective cohort with intensive serial sampling, NT-proBNP increased after each anthracycline cycle, although concentrations varied substantially according to sampling time, highlighting the dynamic nature of natriuretic peptide release during treatment (11). Larger registry-based longitudinal data also showed that increases in NT-proBNP over time were associated with subsequent CTRCD, supporting the concept that biomarker trajectories may capture evolving myocardial stress more effectively than a single pretreatment measurement (18). The frequent NT-proBNP increase observed in the present cohort is therefore consistent with the view that anthracycline exposure can induce early myocardial stress even when baseline LVEF is preserved.

Galectin-3 provides a different biological signal. Unlike NT-proBNP, which is more closely related to wall stress, galectin-3 is linked to inflammation, macrophage activation, extracellular matrix remodeling, and fibrotic signaling (19). This distinction may explain why galectin-3 increased in many patients but showed fewer large relative changes than NT-proBNP. Profibrotic and remodeling pathways may evolve more slowly than hemodynamic stress, may show greater inter-individual heterogeneity, and may not be fully captured within a 4-month treatment window. Prior literature supports this more cautious interpretation: galectin-3 has shown potential associations with subsequent

LVEF and global longitudinal strain (GLS) changes in some prospective anthracycline cohorts, particularly when assessed serially rather than as a single baseline marker (13). However, evidence remains inconsistent, and a meta-analysis found no significant overall pre/post-treatment difference in galectin-3 and only a weak relationship with cardiotoxicity risk, indicating that its role in cardio-oncology surveillance remains less established than that of troponin or natriuretic peptides (20).

The present findings also align with a growing cardio-oncology literature showing that baseline-only assessment may have limited sensitivity for early treatment-related myocardial injury. The 2022 ESC cardio-oncology framework recommends baseline cardiovascular risk stratification and baseline investigations before anthracycline therapy, but it also emphasizes on-treatment surveillance with biomarkers and echocardiography because risk is dynamic and evolves during exposure (21,22). HFA-ICOS-based baseline stratification remains clinically important for organizing surveillance intensity and preventive strategies (23,24). Real-world and multicenter validation studies suggest that higher HFA-ICOS risk categories are associated with greater CTRCD risk, particularly among patients categorized as high or very high risk (21,25). Nevertheless, baseline risk tools do not fully determine which individual patients will develop early biomarker deterioration, and serial functional or biomarker assessment may add information that is not available at pretreatment evaluation alone (23).

This tension between the value of baseline assessment and its limitations is also evident in biomarker and imaging studies. Some cohorts have reported that baseline NT-proBNP or baseline LVEF predicts later dysfunction. In breast cancer patients receiving anthracyclines, higher baseline NT-proBNP predicted greater 6-month LVEF decline in one prospective study (13). In another study, baseline hs-troponin I, older age, lower LVEF, and additional imaging markers predicted subsequent GLS-defined dysfunction (26). Baseline LVEF also predicted later cardiotoxicity in a prospective anthracycline/trastuzumab cohort (11). However, the literature is not uniformly positive. In that same ESC Heart Failure cohort, NT-proBNP did not predict subsequent cardiotoxicity and varied markedly across chemotherapy timepoints (11). Likewise, the CARDIOTOX registry experience, as summarized in later comparative work, found that baseline hs-cTn and NT-proBNP lacked prognostic value for major CTRCD outcomes (23). The negative exploratory findings of the present study therefore fit within a heterogeneous evidence base rather than contradicting a consistent prior signal.

A more consistent message across contemporary studies is that serial changes in biomarkers and myocardial deformation often outperform baseline-only measurements for early detection. In the AIC registry, 3-month increases in troponin T and BNP and relative reductions in LV-GLS were independently associated with subsequent cardiotoxicity (4). In another prospective cohort, a combined strategy using GLS and biomarkers identified early anthracycline-related dysfunction with diagnostic accuracy exceeding 85% (27). Serial GLS decline has repeatedly shown strong predictive performance, with relative reductions greater than 15% emerging as a robust early surveillance signal (23). Additional prospective data showed that baseline GLS alone was insufficient, whereas combining serial imaging, biomarkers, and baseline risk factors improved early detection and risk stratification (28). Similarly, longitudinal observational work demonstrated that worsening GLS predicts subsequent LVEF decline, while prior LVEF does not reliably predict later GLS deterioration (29). Together, these findings support the concept that anthracycline cardiotoxicity surveillance is inherently dynamic and that clinically useful risk information often emerges after treatment initiation.

Within this context, the present results have several clinical implications. First, baseline assessment remains essential because it identifies patients with pre-existing cardiovascular vulnerability and provides the foundation for structured surveillance according to established risk frameworks such as HFA-ICOS (22,25). Second, preserved baseline LVEF, near-normal diastolic filling indices, and low baseline NT-proBNP or galectin-3 should not be interpreted as excluding the possibility of early biomarker worsening during anthracycline therapy. Third, increases in NT-proBNP or galectin-3 should be interpreted cautiously and in combination with symptoms, ECG findings, troponin, renal function, and imaging findings, particularly GLS, which currently has stronger evidence for early detection than biomarker changes alone (23,24). The descriptive relative-change thresholds used in the present study are useful for illustrating the frequency and magnitude of biomarker deterioration in an untreated observational setting, but they should not be interpreted as validated diagnostic cut-offs or used to define cardiotoxicity.

This study has several strengths. By focusing only on placebo-treated participants, it provides a relatively clear natural-history view of early biomarker behavior during anthracycline exposure without confounding by active cardioprotective treatment. All included patients had complete paired baseline and 4-month NT-proBNP and galectin-3 data, minimizing missing-outcome bias within the analyzed cohort. The analysis also focused deliberately on routinely available baseline predictors, which makes the findings relevant to real-world oncology and cardio-oncology settings where advanced imaging or broad biomarker panels may not always be available. Finally, the simultaneous assessment of NT-proBNP and galectin-3 allowed evaluation of two biologically complementary pathways: myocardial wall stress and remodeling/fibrotic activation.

Several limitations should temper interpretation. This was an exploratory secondary analysis with a small sample size from a single center, limiting statistical power and increasing the possibility of type II error, particularly for modest predictor–outcome associations. The cohort was predominantly female and likely enriched for breast cancer, which may limit generalizability to other malignancies, anthracycline regimens, and higher-risk cardiovascular populations. Follow-up was limited to 4 months, and the outcomes were biomarker changes rather than symptomatic heart failure, formally adjudicated CTRCD, GLS decline, or long-term LVEF deterioration. No adjustment was made for multiple comparisons, and the regression analyses were simple exploratory models rather than multivariable prediction models with internal or external validation. In addition, galectin-3 values were reported in pg/mL, so cross-study comparisons require caution because assay platforms, reporting conventions, and units vary across the literature. These limitations mean that the present results should be viewed as hypothesis-generating rather than definitive evidence against the value of baseline risk stratification.

Future research should therefore move beyond baseline-only prediction. Larger prospective cohorts with longer follow-up are needed to determine whether early deterioration in NT-proBNP and galectin-3 predicts later CTRCD, symptomatic heart failure, treatment interruption, or persistent ventricular dysfunction. The most promising approach is likely dynamic risk modeling that integrates baseline clinical risk, serial troponin and natriuretic peptide measurements, galectin-3 or other remodeling markers, and deformation imaging such as GLS (4,28,30). Emerging evidence also suggests that multimarker approaches may outperform single biomarkers, although the optimal biomarker combination, timing of measurement, and clinically actionable thresholds remain unsettled (20,31,32).

In conclusion, this placebo-arm exploratory analysis suggests that early biomarker deterioration is common during anthracycline chemotherapy, particularly for NT-proBNP, even in patients with preserved baseline cardiac function. Routine baseline clinical, echocardiographic, and biomarker characteristics showed limited ability to identify which patients would later exhibit greater NT-proBNP or galectin-3 worsening. These findings support a cautious, hypothesis-generating conclusion: baseline evaluation remains necessary, but baseline-only profiling may be insufficient, and serial surveillance with dynamic risk assessment during anthracycline therapy may provide more clinically informative monitoring.

DECLARATIONS

Ethics approval and consent to participate

The parent randomized clinical trial received ethical approval from the Ethics Committee of the College of Medicine, Hawler Medical University, and from the Duhok General Directorate of Health Ethics Committee. All participants provided written informed consent before enrollment. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The present secondary analysis used data collected within the approved parent trial and was limited to placebo-treated participants with complete baseline and follow-up biomarker data.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available because they contain participant-level clinical data, but they may be available from the corresponding author upon reasonable request and subject to institutional and ethical approval.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HAS contributed to study conception and design, participant recruitment, data collection, laboratory coordination, statistical analysis, data interpretation, and drafting of the manuscript. NAMA contributed to study supervision, methodology, data interpretation, and critical revision of the manuscript. ROT contributed to clinical supervision, participant recruitment, oncology-related data interpretation, and critical revision of the manuscript. All authors read and approved the final manuscript.

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Trial registration

The parent randomized clinical trial was registered at ClinicalTrials.gov: NCT06888505.

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