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ORIGINAL RESEARCH

Cardiotoxicity and Cardiovascular Biomarkers in Patients With Breast Cancer: Data From the GeparOcto-GBG 84 Trial

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BACKGROUND: Patients with breast cancer can be affected by cardiotoxic reactions through cancer therapies. Cardiac biomarkers, like NT-proBNP (N-terminal pro-B-type natriuretic peptide) and high-sensitivity cardiac troponin T, might have predictive value.

METHODS AND RESULTS: Echocardiography, ECG, hemodynamic parameters, NT-proBNP and high-sensitivity cardiac troponin T were assessed in 853 patients with early-stage breast cancer randomized in the German Breast Group GeparOcto-GBG 84 phase III trial. Patients received neo-adjuvant dose-dense, dose-intensified epirubicin, paclitaxel, and cyclophosphamide (id-dEPC group, n=424) or paclitaxel, non-pegylated doxorubicin, and in triple negative breast cancer, (paclitaxel, non-pegylated doxorubicin, carboplatin group, n=429) treatment for 18 weeks. Patients positive for human epidermal growth receptor 2 (n=354, 41.5%) received monoclonal antibodies on top of allocated therapy; 119 (12.9%) of all patients showed a cardiotoxic reaction during therapy (15 [1.8%] using a more strict definition). Presence of cardiotoxic reactions was irrespective of treatment allocation (P=0.31). Small but significant increases in NT-proBNP developed early in patients with a cardiotoxic reaction as compared with those without in whom NT-proBNP rose only towards the end of therapy (P=0.04). High-sensitivity cardiac troponin T rose early in both groups. Logistic regression showed that NT-proBNP (odds ratio [OR], 1.03; 95% CI, 1.008–1.055; P=0.01) and hemoglobin (OR, 1.31; 95% CI, 1.05–1.63; P=0.02) measured at 6 weeks after treatment initiation were significantly associated with cardiotoxic reactions.

CONCLUSIONS: NT-proBNP and hemoglobin are significantly associated with cardiotoxic reactions in patients with early-stage breast cancer undergoing dose-dense and dose-intensified chemotherapy, but high-sensitivity cardiac troponin T is not.

REGISTRATION: URL: http://www.clinicaltrials.gov; Unique identifier: NCT02125344.

Key Words: biomarker ■ breast cancer ■ cardio-oncology ■ cardiotoxicity ■ left ventricular ejection fraction

Breast cancer is increasing in prevalence and affects currently >3.3 million women in the United States alone.[1] Estimates assume that 2.1 million new cases will be diagnosed per year worldwide. Indeed, 24.2% of all cancers in women are attributed to breast cancer, making it the most common cancer in women.[2] Approximately 15% to 30% of patients have an amplification of the human epidermal growth factor receptor 2 (HER2). HER2 expression is associated with tumor and nodal growth, metastasis at an early stage, and in

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CLINICAL PERSPECTIVE

What Is New?

- NT-proBNP (N-terminal pro-B-type natriuretic peptide) and hemoglobin are strongly associated with cardiotoxic reactions in patients with breast cancer undergoing chemotherapy.
- Patients with breast cancer develop anemia and iron deficiency during therapy.

What Are the Clinical Implications?

- Assessment of hemoglobin and NT-proBNP during chemotherapy may help in the identification of patients at risk, particularly at 6 weeks of therapy initiation.

Nonstandard Abbreviations and Acronyms

EOT	end of therapy
HER2	human epidermal growth receptor 2
hs-cTnT	high-sensitivity cardiac troponin T
PM(Cb)	paclitaxel, non-pegylated doxorubicin, carboplatin
TSAT	transferrin saturation

general high morbidity and mortality.[3–6] Chemotherapy regimens have traditionally included anthracyclines such as doxorubicin and epirubicin, and, more recently, targeted therapies like the HER2-directed monoclonal antibodies trastuzumab, pertuzumab for patients positive with HER2, and trastuzumab emtansine.[4,6–9] Improvement of therapy has yielded better survival, but these advances are often associated with cardiovascular toxicities. Indeed, anthracyclines and HER2 antibodies have been associated with the development of cardiac dysfunction or even overt heart failure in up to 34% of all cases.[5] Additional risk factors include a short time interval between anthracycline treatment and anti-HER2 therapies, and underlying cardiovascular comorbidities such as arterial hypertension, a pre-existing reduced left ventricular ejection fraction (LVEF) and advanced age in general.[1,5,6] The main difference between the cardiotoxicities of anthracyclines and anti-HER2 therapies is that only the latter is potentially reversible, while the first is usually irreversible.[1,10–13]

Cardio-oncology is a currently evolving field that aims to assess and treat cardiovascular perturbations in patients with cancer. One major field is the development of an understanding of risk factors for cardiotoxic reactions in the course of cancer chemotherapy, because these remain poorly defined. Other areas embrace the development of cardiovascular problems irrespective of chemotherapy[14,15] and the development of cancer in patients with heart failure.[16] Especially cardiotoxic predicting biomarker, as NT-proBNP (N-terminal pro-B-type natriuretic peptide), and the troponins, seem to have prognostic relevance and gain further interest.[1,16] Herein, we sought to investigate the prevalence of cardiotoxic reactions among women undergoing therapy regimens for breast cancer within the GeparOcto trial.[17] We further aimed to establish predictors of cardiotoxic reactions while undergoing the aforementioned treatments.

METHODS

Study Population

Between December 2014 and June 2016, the German Breast Group GeparOcto-GBG 84 trial (phase III) randomized 1:1961 patients with early stage breast cancer to neo-adjuvant dose-dense, dose-intensified epirubicin, paclitaxel, and cyclophosphamide (iddEPC) group treatment or to weekly paclitaxel in combination with non-pegylated doxorubicin, and carboplatin (PM(Cb) group) treatment for 18 weeks, of whom 945 started treatment. The study was approved by the responsible ethics committee of the medical faculty Heidelberg and patients gave written informed consent. The trial is registered with ClinicalTrials.gov number NCT02125344. Patients in the iddEPC group received epirubicin (150 mg/m^2) every other week for 3 cycles, followed by paclitaxel (P, 225 mg/m^2) every other week also for 3 cycles and then cyclophosphamide (C, 2000 mg/m^2), likewise every other week for 3 cycles. Patients in the PM(Cb) group received paclitaxel (80 mg/m^2) weekly in combination with non-pegylated liposomal doxorubicin (20 mg/m^2) weekly. Patients with triple negative breast cancer (negative for the expression of HER2, estrogen receptor, progesterone receptor) received additional carboplatin (AUC, 1.5) weekly for 18 weeks. Patients positive with HER2 received trastuzumab (6 mg/kg) every 3 weeks and pertuzumab (420 mg) every 3 weeks, in parallel to all chemotherapy cycles except to epirubicin (iddEPC group), according to the regimen depicted in Figure 1. For both antibodies, a loading dose was applied at the beginning of therapy.[17]

Main criteria for inclusion were centrally confirmed (biopsy) unilateral or bilateral carcinoma of the breast, lesion with palpable size ≥2 cm or sonographically confirmed size ≥1 cm, tumor stage cT1c–cT4a-d. Patients with luminal-B-like tumors (estrogen receptor and/or progesterone receptor ≥1%, HER2-negative, Ki67 >20%) were eligible only in the case of histologically confirmed positive nodal status. In addition, patients were required to have the following criteria fulfilled at baseline: normal LVEF (>55%) on transthoracic echocardiography, no known cardiac disease,

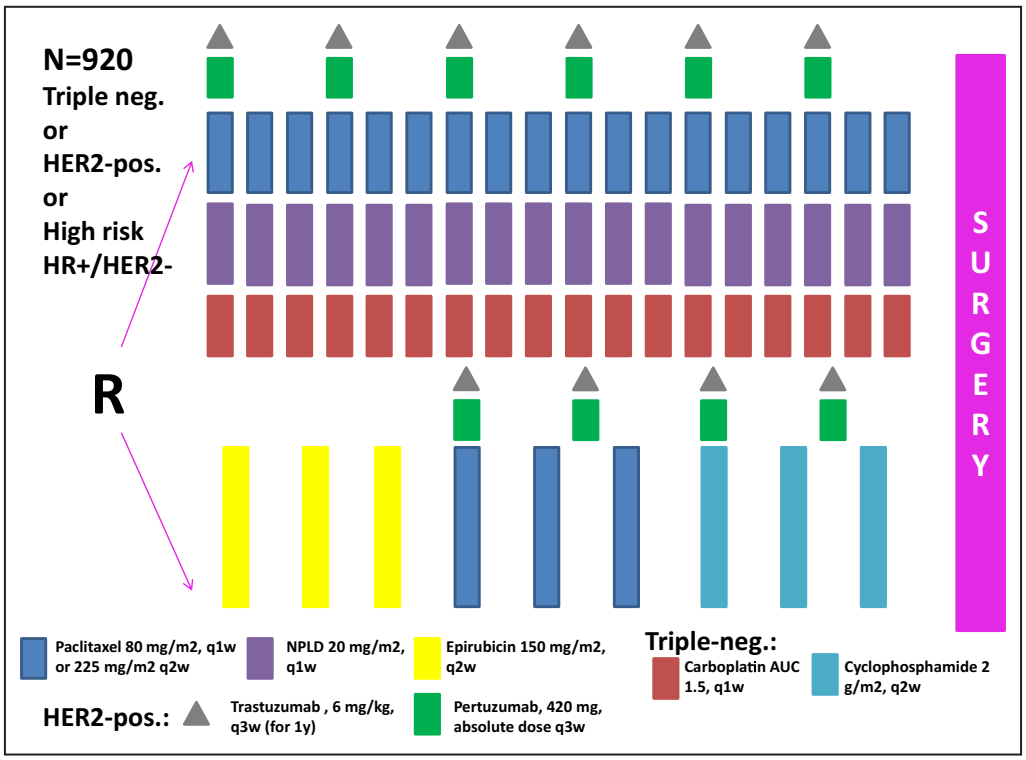

Figure 1. Study design GeparOcto-GBG 84 trial (phase III).
HER2 indicates human epidermal growth receptor 2; iddEPC, neo-adjuvant dose-dense, dose-intensified epirubicin, paclitaxel, and cyclophosphamide; NPLD, non-pegylated doxorubicin; R, randomization; and PM(Cb), paclitaxel, non-pegylated doxorubicin, carboplatin.

Karnofsky performance index ≥90%; absolute neutrophil ≥2.0×10^9/L, platelet count ≥100×10^9/L, and hemoglobin ≥10 g/dL, transaminases and bilirubin ≤1.5 times upper reference limit and alkaline phosphatase ≤2.5 times upper reference limit, and age ≥18 years. The main exclusion criteria included any previous chemotherapy and any previous radiotherapy for breast cancer. Likewise, pregnant or lactating women were excluded as well as those with severely reduced general status, previous cancer within <5 years, known or suspected heart failure, coronary artery disease, angina, history or electrocardiographic signs of previous myocardial infarction, uncontrolled hypertension, treatment with ≥2 antihypertensive drugs, cardiac arrhythmia with continued treatment, significant valvular disease, or acute infection.[17] All data are on file at German Breast Group, Neu-Isenburg, Germany and can be made available upon request addressed to abstracts@gbg.de.

Cardiovascular Investigations and Cardiac Biomarkers

Of 961 patients included in the GeparOcto trial, all required cardiovascular investigations were available in at least 1 time-point in addition to baseline in 853 patients (Figure 2). These formed the basis for the present analysis, and patients' baseline characteristics were similar for the overall cohort of 961 patients. Serum samples were available for all 853 patients at baseline, for 812 patients at 6 weeks of follow-up, and for 690 patients for all time-points (baseline, 6 weeks, and end of therapy [EOT]). At baseline, 6 weeks after treatment initiation, and at EOT all patients underwent a thorough cardiovascular assessment that included transthoracic echocardiography for LVEF assessment, 12-lead ECG, measurement of blood pressure and heart rate. Serum and plasma samples were withdrawn from an antecubital vein and immediately frozen at −20°C. Samples were then transferred to a central biobank for storage at −80°C for later analysis. The following blood biomarkers were thus assessed from serum samples: NT-proBNP, high-sensitivity cardiac troponin T (hsTrop T), serum ferritin, and transferrin saturation. Assays for NT-proBNP and hsTrop T for use on the Elecsys were kindly provided free of charge by Roche Diagnostics (Basel, Switzerland).

Study End Points

Cardiotoxic reactions were defined as a decrease in LVEF ≥10 percentage points from baseline to any time during therapy (primary end point). As a second, more rigid, definition a decrease in LVEF ≥10 percentage

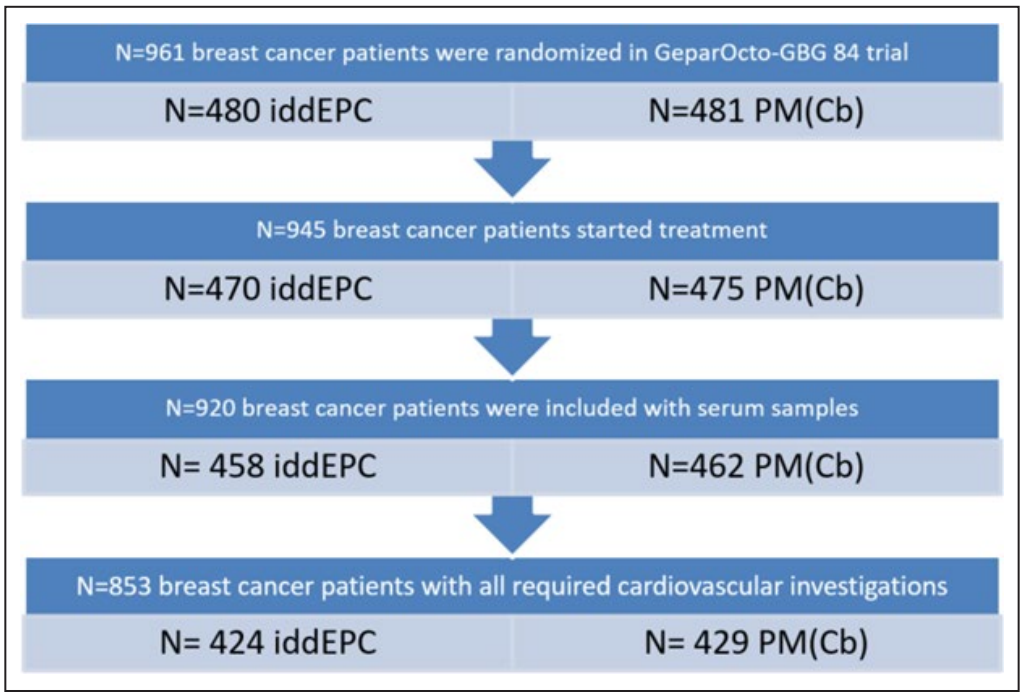

Figure 2. Consort statement GeparOcto-GBG 84 trial (phase III).
iddEPC indicates neo-adjuvant dose-dense, dose-intensified epirubicin, paclitaxel, and cyclophosphamide; and PM(Cb), paclitaxel, non-pegylated doxorubicin, carboplatin.

points from baseline to any time during therapy in patients with an LVEF ≤50% was used. Secondary end points included the incidence and prevalence of iron deficiency, defined as transferrin saturation (TSAT) <20% as well as the incidence and prevalence of anemia, defined according to World Health Organization criteria as hemoglobin <12 g/dL.[18]

Statistical Analysis

Data are presented as means with SD, for continuous variables or numbers (n) and percentage (%), for categorical parameters. Student unpaired and paired *t*-tests and the Chi-square test were used as appropriate. Assessment of the primary outcome was done using univariate and multivariate logistic regression models. To analyze the cardiotoxic reaction predictability of hemoglobin and the cardiac biomarkers at week 6, after trends were noted using univariate logistic regression models, a multivariate logistic regression model was conducted. The multivariate logistic regression model was adjusted for demographic factors, comorbidities and serum-creatinine. Simple regression analyses were conveyed to analyze the correlation, using the standardized regression coefficient, of hemoglobin and TSAT with demographic and laboratory parameters at baseline. A value of $P<0.05$ was considered statistically significant. No multiplicity adjustment was applied. All analyses were performed using the Statistical Package for the Social Sciences version 25 (IBM, New York, United States of America) and JASP open source package 0.11.1 (Amsterdam, Netherlands).

RESULTS

A total of 853 patients formed the basis of the present analysis; 424 (49.7%) were allocated to the iddEPC and 429 (50.3%) to the PM(Cb) treatment group. All patients had high-risk early breast cancer (4.9% cT3, 1.4% cT4a-c, 2.2% cT4d, 66.9% G3 tumors) and median age was 48.0±10.4 with a median body mass index of 25.7±5.0 kg/m^2. The majority (61.8%) of patients were premenopausal. Baseline variables are shown in Table 1; these were well balanced between treatment groups. A total of 354 (41.5%) patients were HER2-positive.

Baseline Assessments

At baseline, all patients had an LVEF of ≥55%, as defined in the inclusion criteria. The mean LVEF was 65.5±5.9%, the systolic blood pressure and the heart rate were in the normal range as well (Table 1). At baseline, 53 (6.2%) patients were anemic; 295 patients (34.6%) fulfilled the criterion of iron deficiency. Patients' mean NT-proBNP at baseline was 82.1±62.8 pg/mL; a value >125 pg/mL was noted in 141 (16.5%) patients at baseline with 66 in the iddEPC and 75 in the PM(Cb) group ($P=0.45$). Only 3 patients had elevated hsTrop T level at baseline, defined as ≥14 ng/L, mean hsTrop T was 3.40±1.41 ng/L (Table 1).

Table 1. Baseline Characteristics Cardiotoxicity vs Non-Cardiotoxicity

Variable	All (n=853)	Cardiotoxicity (n=119)	Non-Cardiotoxicity (n=734)	*P* Value*
Age, y	47.9±10.4	48.8±10.3	47.8±10.4	0.35
BMI, kg/m^2	25.7±5.0	25.7±4.9	25.7±5.1	0.98
Ki67, %	49.4±21.7	50.4±22.1	49.2±21.6	0.57
Karnofsky Index 100, n (%)	778 (90.98%)	109 (91.58%)	667 (90.87%)	0.80
Premenopausal, n (%)	527 (61.8%)	73 (61.3%)	454 (61.9%)	0.92
ER and/or PgR positive	383 (44.9%)	55 (46.2%)	328 (44.7%)	0.76
HER2+	354 (41.5%)	49 (41.2%)	305 (41.6%)	0.94
LVEF, %	65.5±5.9	72.2±6.7	64.4±5.0	<0.001
Blood pressure (systolic), mm Hg	123.5±11.6	123.5±11.2	123.5±11.6	0.94
Blood pressure (diastolic), mm Hg	78.0±8.2	78.4±8.6	77.8±8.3	0.47
Pulse, /min	75.4±10.5	75.3±10.8	75.4±10.4	0.95
Cardiovascular medication				
ACE inhibitors	56 (6.6%)	4 (3.4%)	52 (7.1%)	0.13
Aspirin	9 (1.1%)	2 (1.7%)	7 (1.0%)	0.47
Diuretics	14 (1.6%)	3 (2.5%)	11 (1.5%)	0.42
NSAID	26 (3.0%)	2 (1.7%)	24 (3.3%)	0.35
ß-blocker	85 (10.0%)	15 (12.6%)	70 (9.5%)	0.30
Cardiovascular comorbidities				
Hypertension	141 (16.5%)	16 (13.4%)	125 (17.0%)	0.33
Dyslipidemia	19 (2.2%)	4 (3.4%)	15 (2.0%)	0.37
Diabetes mellitus	20 (2.3%)	3 (2.5%)	17 (2.3%)	0.89
Laboratory				
NT-proBNP, pg/mL	82.1±62.8	82.8±76.8	82.0±60.3	0.91
NT-proBNP >125 pg/mL, n (%)	141 (16.5%)	18 (15.1%)	123 (16.8%)	0.66
hsTrop T, ng/L	3.40±1.41	3.43±1.78	3.39±1.34	0.76
Ferritin, µg/L	105.869±98.07	109.899±101.09	105.214±97.63	0.63
TSAT, %	23.8±10.0	25.1±11.8	23.6±9.7	0.14
TSAT <20%	295 (34.6%)	39 (32.8%)	256 (34.9%)	0.65
Transferrin, mg/dL	254.0±40.1	257.9±45.1	253.4±39.2	0.26
Creatinine, mg/dL	0.73±0.13	0.75±0.12	0.72±0.13	0.07
Hemoglobin , g/dL	13.5±1.0	13.5±1.0	13.5±1.0	0.66
Hemoglobin <12, g/dL	53 (6.2%)	7 (5.9%)	46 (6.3%)	0.88
Platelets,/nL	269.2±63.6	267.7±55.2	269.7±65.0	0.78
Lymphocytes,/nL	2.57±3.95	3.05±5.38	2.50±3.66	0.16
WBC,/nL	7.51±2.10	7.50±2.07	7.51±2.11	0.93
ANC,/nL	5.17±4.91	4.76±1.72	5.23±5.25	0.33

Values are mean±SD, %, or n. ACE indicates angiotensin-converting enzyme; ANC, absolute neutrophil count; BMI, body mass index; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; hsTrop T, high-sensitivity cardiac troponin T; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal brain natriuretic peptide; PgR, progesterone receptor; TSAT, transferrin saturation; and WBC, white blood cells.

*Analysis of student *t*-test or Chi-square *P* value (patients with cardiotoxicity vs patients without cardiotoxicity).

Change in Cardiovascular Function Parameters During Therapy

In the overall population, mean LVEF remained rather stable from baseline (65.5±5.9%) to 6 weeks (65.4±6.1%) and to the end of therapy (64.7±6.3%). Blood pressure rose from 123.5±11.6 mm Hg to 137.21±14.36 mm Hg during therapy and pulse from 75.4±10.5/min to 91.64±12.27/min during therapy. There were no significant differences between the cardiotoxicity groups or treatment arms. Irrespective of treatment allocation, a total of 119 (12.9%) patients were found to fulfill the criteria of a cardiotoxic reaction defined as a decrease of LVEF of ≥10 percentage points at any time during therapy. Mean LVEF in

this group decreased from 72.2±6.7% at baseline to 63.7±8.2% at 6 weeks (*P*<0.001 versus baseline) to 61.8±8.3% at the end of therapy (*P*<0.001). The mean decrease in LVEF from baseline to end of therapy was 13.3 percentage points. In the group without a cardiotoxic reaction, no statistically significant difference was noted at any time-point compared with the baseline assessment (baseline, 64.5±5.0%; week 6, 65.7±5.7%; end of therapy, 65.2±5.7%), as was expected according to the definition. Of all patients with a cardiotoxic reaction, 115 (96.4%) patients had anemia during therapy and only 4 (3.36%) patients were without anemia. Using the stricter definition of LVEF drop by 10 percentage points together with a value ≤50%, 15 (1.8%) patients had a cardiotoxic reaction. Mean LVEF in this group decreased from 63.5±5.8% at baseline to 56.8±9.3% at 6 weeks (*P*<0.001 versus baseline) to 48.7±7.4% at the end of therapy (*P*<0.001).

Biomarkers

In the overall population, mean NT-proBNP did not change from baseline (82.1±62.8 pg/mL) to 6 weeks (84.6±93.6 pg/mL, *P*=0.3), but showed a modest though statistically significant increase towards the end of therapy (97.3±129.0 pg/mL, *P*<0.001 versus baseline, Figure 3A). At baseline, there was a small, but statistically significant difference between both treatment groups (iddEPC: 76.8±57.2 pg/mL versus PM(Cb) 87.4±67.4 pg/mL, *P*=0.01). Such differences were also present at 6 weeks (iddEPC: 117.5±112.8 pg/mL versus PM(Cb): 52.9±54.4 pg/mL, *P*<0.001) and at the end of therapy (iddEPC: 110.9±116.9 pg/mL versus PM(Cb): 82.9±139.3 pg/mL, *P*=0.005). As compared with the PM(Cb) arm, NT-proBNP remained significantly elevated throughout therapy in the iddEPC arm (both *P*<0.001 versus baseline, Figure 3B). In the PM(Cb) group,

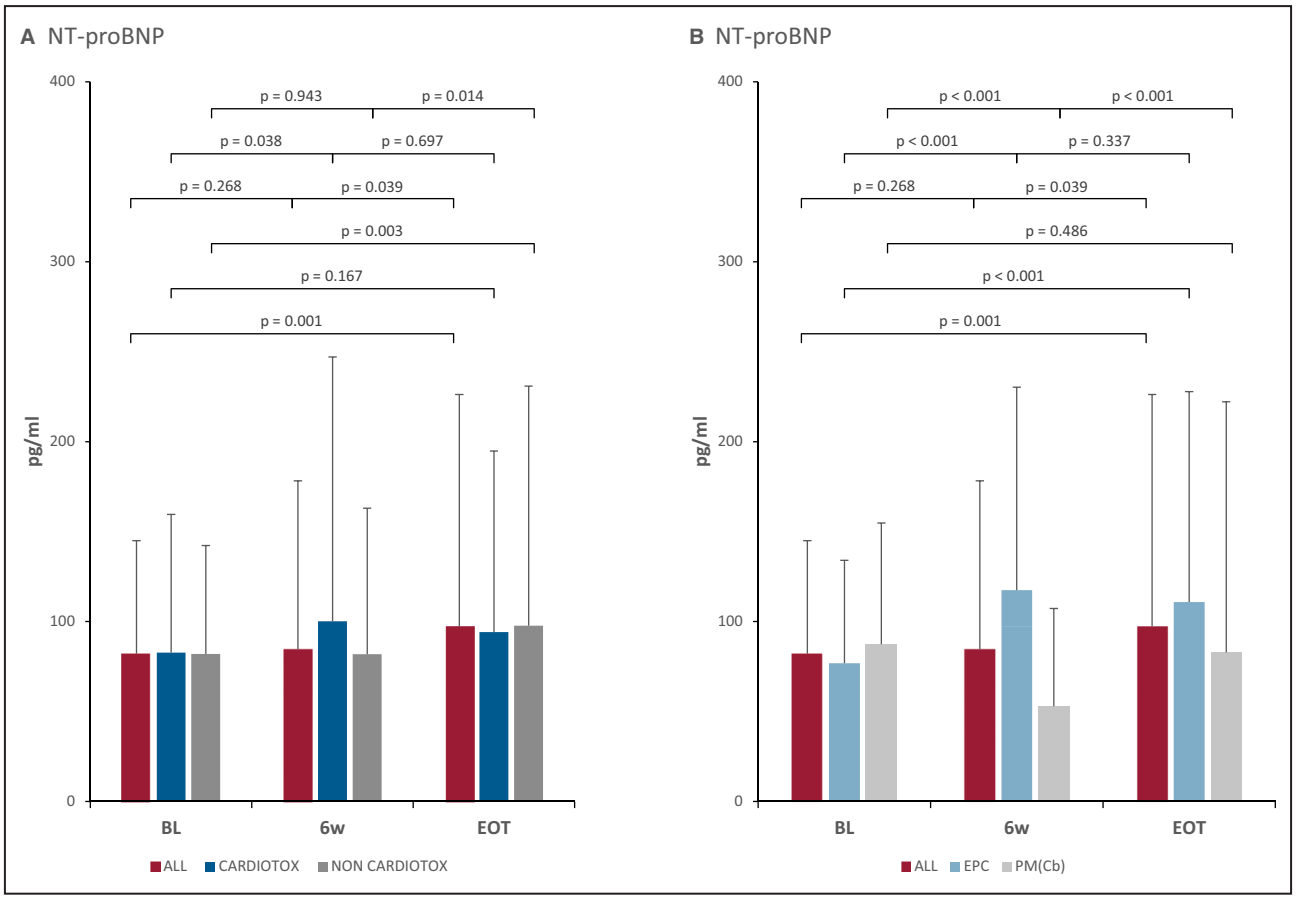

Figure 3. NT-proBNP during therapy.
A, NT-proBNP (N-terminal pro-B-type natriuretic peptide) (cardiotoxicity vs non-cardiotoxicity), (**B**) NT-proBNP (neo-adjuvant dose-dense, dose-intensified epirubicin, paclitaxel, and cyclophosphamide vs paclitaxel, non-pegylated doxorubicin, carboplatin). NT-proBNP as assessed by all (red), cardiotoxicity (blue bar) vs non-cardiotoxicity (grey bar) and between all (red), epirubicin, paclitaxel, cyclophosphamide (light blue bar) and paclitaxel, non-pegylated doxorubicin, carboplatin (light grey bar) group. Analysis is shown as mean±SD and independent samples t-test shown by *P* values. The time points of assessment are baseline, after 6 weeks, and at the end of therapy. iddEPC indicates neo-adjuvant dose-dense, dose-intensified epirubicin, paclitaxel, and cyclophosphamide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and PM(Cb), paclitaxel, non-pegylated doxorubicin, carboplatin.

NT-proBNP decreased significantly during therapy until week 6 (P<0.001 versus baseline). Patients with a cardiotoxic reaction had a modest elevation in NT-proBNP of 100.2±146.8 pg/mL as compared with patients without a cardiotoxic reaction (81.9±81.2 pg/mL), apparent after 6 weeks (P=0.06). Both patient groups started with similar NT-proBNP values (baseline cardiotoxicity: 82.8±76.8 pg/mL versus non-cardiotoxicity 82.0±60.3). In the group of patients with cardiotoxicity, the elevation in NT-proBNP was significant at 6 weeks (P=0.038 versus baseline, Figure 3A). Using the more rigid definition for cardiotoxicity that included only patients whose LVEF dropped ≤50%, NT-proBNP was 101.3±80.0 pg/mL at baseline, 91.0±84.2 pg/mL at week 6, and 133.5±144.8 pg/mL at end of therapy (P=0.4 versus baseline) for the cardiotoxic group. In the non-cardiotoxic group, NT-proBNP remained stable with a slight elevation towards the end of therapy (baseline, 81.8±62.4; week 6, 84.5±93.9; end of therapy, 96.5±128.7; P=0.002). hsTrop T increased from baseline to 6 weeks of cancer therapy among the cardiotoxic group (baseline, 3.40±1.41 ng/L; 6 weeks, 6.45±4.95 ng/L; P<0.001) and stayed stable until the end of therapy 6.42±4.23 ng/L (Figure 4A). Overall 3 (0.4%) patients had elevated hs Trop T values at baseline, 56 (7.39%) patients after 6 weeks and 39 (5.9%) patients at the end of therapy. Similar findings were seen for the stricter definition (baseline, 3.47±1.23 ng/L; 6 weeks, 6.99±5.35 ng/L; end of therapy, 7.17±4.88 ng/L). hsTrop T showed significant differences between end of therapy and baseline in the cardiotoxic group according to the stricter definition (P=0.02). After 6 weeks of therapy, the hsTrop T values were significantly higher in the iddEPC treatment group versus PM(Cb) (9.19±5.54 ng/L versus 3.85±2.20 ng/L; P<0.001). This difference remained statistically significant also at the end of therapy (iddEPC, 7.27±4.17 ng/L versus PM(Cb), 5.54±4.12 ng/L; P<0.001, Figure 4B).

Iron Deficiency and Anemia

Mean hemoglobin decreased during therapy in all patients, starting at 13.5±1.0 g/dL to 11.2±1.0 g/dL at week 6 after start of chemotherapy to 10.5±1.2 g/dL at the end of therapy (both P<0.001 versus baseline). A similar hemoglobin behavior was found in the cardiotoxic group and in the non-cardiotoxic group (Figure 5A), however, hemoglobin was significantly different between the 2 groups at week 6 (P=0.03, Figure 5A). No difference was noted when applying the stricter definition of cardiotoxicity with patients whose LVEF dropped to ≤50%. Using this definition there were significant differences for hemoglobin between all points in time (baseline, P<0.001; 6 weeks, P=0.022; EOT, P<0.001). Differences were statistically significant between treatment arms at week 6 (iddEPC, 10.9±1.0 g/dL versus PM(Cb), 11.6±1.0 g/dL; P<0.001) and at end of therapy (iddEPC, 10.3±1.2 g/dL versus PM(Cb), 10.7±1.2 g/dL; P<0.001). As depicted in Figure 5B, both decreased over time throughout the treatment period.

At baseline, 295 (34.6%) patients were iron deficient. During treatment, 59 patients at week 6 and 48 patients at end of therapy with a cardiotoxic reaction showed iron deficiency (baseline, 32.8%; 6 weeks, 52.2%; EOT, 51.1%). The corresponding value for patients with iron deficiency without a cardiotoxic reaction were 328 patients at week 6 and 335 patients at end of therapy (baseline, 34.9%; 6 weeks, 49.6%; EOT, 58.8%). Mean TSAT values for all patients decreased over time. Differences were significant between treatment arms at week 6 (iddEPC, 25.8±16.2% versus PM(Cb), 19.2±9.3%; P<0.001) and at the end of therapy (iddEPC, 23.3±15.3% versus PM(Cb), 18.9±10.2%; P<0.001). TSAT at baseline for patients with a cardiotoxic reaction was 25.1±11.8%, 23.1±15.2% at week 6, and 21.7±15.1% at the end of therapy (all not significantly different from the non-cardiotoxic group). TSAT values for patients with a cardiotoxic reaction applying the stricter definition of cardiotoxicity with patients whose LVEF dropped to ≤50% were 20.8±6.0%, 21.3±14.9% at week 6, and 19.8±16.4% at the end of therapy. TSAT values in the patient group without a cardiotoxic reaction were 23.9±10.1% at baseline, 22.5±13.5% at week 6, and 21.2±13.2% at the end of therapy. No major difference was noted for patients without a cardiotoxic reaction whether the original or the more strict definition of cardiotoxicity was used. Using simple regression analysis, we found that TSAT correlated with body mass index (R=−0.14, P<0.001), hemoglobin (R=0.24, P<0.001), and ferritin (R=0.21, P<0.001), but not with age (R=0.06, P=0.099), leukocytes (R=−0.03, P=0.4), or serum creatinine (R=0.05, P=0.1). Hemoglobin values did not correlate with body mass index (R=0.04, P=0.3), but with leukocytes (R=0.19, P<0.001), serum creatinine (R=0.1, P=0.004), and ferritin (R=0.2, P<0.001).

Prognostic Markers for Cardiotoxic Reactions

Using baseline variables entered into a univariable logistic regression model, we found that none of the baseline variables were significantly associated with a cardiotoxic reaction. This was true for age, body mass index, all blood biomarkers and histological breast cancer subtypes, for all available cardiovascular risk factors, for patients' cardiovascular medication as well as for all cardiovascular functions parameters. A trend was noted for serum creatinine

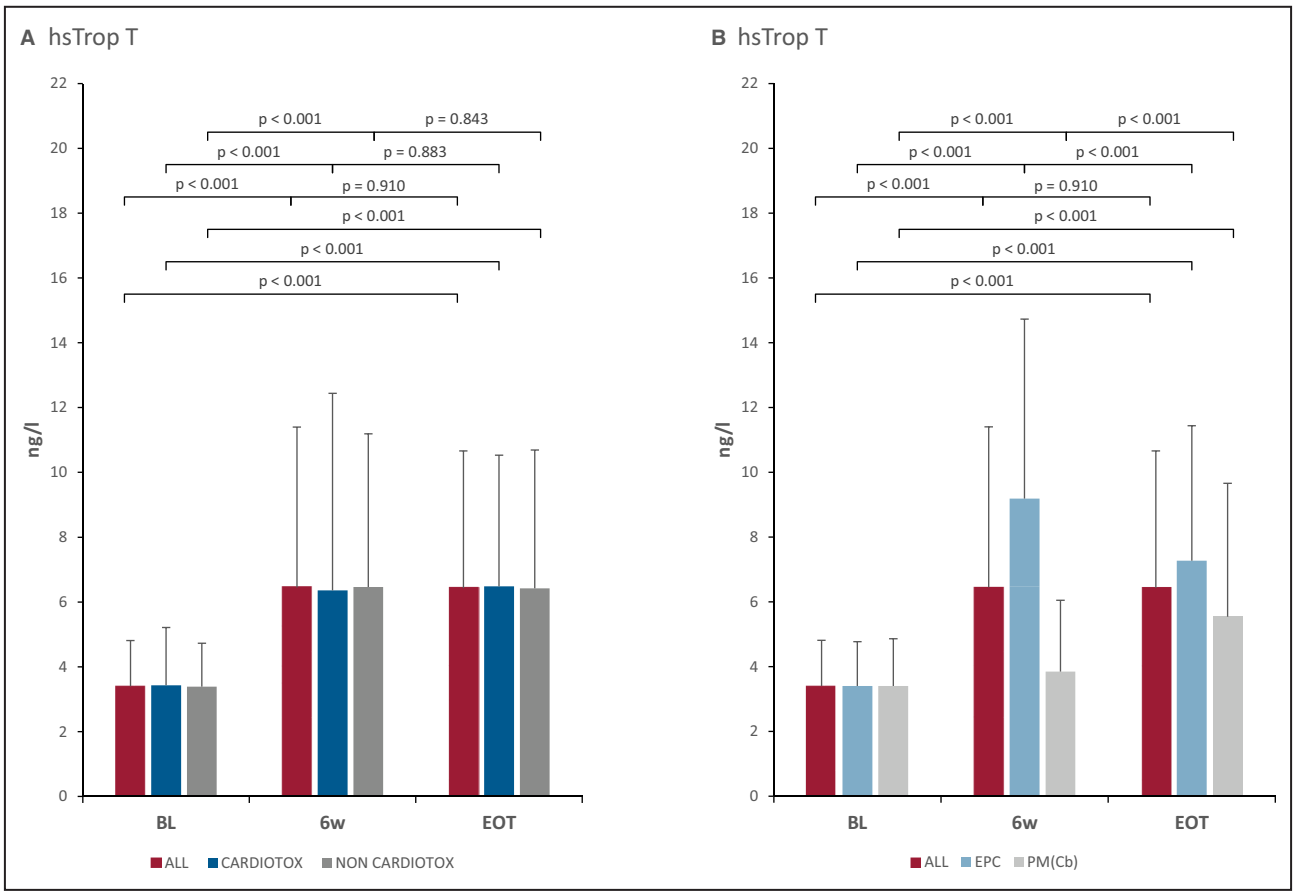

Figure 4. High-sensitivity cardiac troponin T during therapy.
A, High-sensitivity cardiac troponin T (hsTrop T) (cardiotoxicity vs non-cardiotoxicity); (**B**) High-sensitivity cardiac troponin T (neo-adjuvant dose-dense, dose-intensified epirubicin, paclitaxel, and cyclophosphamide vs paclitaxel, non-pegylated doxorubicin, carboplatin). High-sensitivity cardiac troponin T as assessed by all (red), cardiotoxicity (blue bar) vs non-cardiotoxicity (grey bar) and between all (red), EPC (light blue bar) and PM(Cb) (light grey bar) group. Analysis is shown as mean±SD and independent samples t-test shown by *P* values. The time points of assessment are baseline, after 6 weeks, and at the end of therapy. iddEPC indicates neo-adjuvant dose-dense, dose-intensified epirubicin, paclitaxel, and cyclophosphamide; hsTrop T, high-sensitivity cardiac troponin T; and PM(Cb), paclitaxel, non-pegylated doxorubicin, carboplatin.

(odds ratio [OR], 3.74; 95% CI, 0.89–15.6; *P*=0.07, Table 2). This situation changed when parameters were assessed at 6 weeks of follow-up and were entered into the model. We found that hemoglobin (OR, 1.23; 95% CI, 1.02–1.49; *P*=0.03) and LVEF at 6 weeks were significantly associated with cardiotoxic reactions (Table 2). A trend was noted for NT-proBNP at week 6 (OR, 1.02; 95% CI, 1.00–1.035; *P*=0.07; Table 2). In a multivariable model adjusted for demographic factors, comorbidities, and serum creatinine, hemoglobin (OR, 1.31; 95% CI, 1.05–1.63; *P*=0.02) and NT-proBNP (OR, 1.03; 95% CI, 1.008–1.055; *P*=0.01) at 6 weeks showed significant associations with cardiotoxic reactions (Table 2). Using the stricter definition and logistic regression analysis, we found that hemoglobin at the end of therapy (OR, 1.64; 95% CI, 0.03–0.96; *P*=0.037) was significantly associated with cardiotoxic reactions.

DISCUSSION

Cardiotoxic reactions, defined as LVEF reductions, were highly prevalent among patients receiving treatment for breast cancer in the GeparOcto trial, affecting 12.9% of all patients randomized in the trial. As expected, anemia was highly prevalent during therapy, affecting 96.6% of all patients with a cardiotoxic reaction. Prevalence values varied according to the definition of cardiotoxicity applied between the aforementioned 12.9% and 1.8% using the more strict definition that included only patients whose LVEF dropped to ≤50%. Iron deficiency was present in 34.6% of all patients at baseline and 49.9% of all patients at 6 weeks, and 57.7% at end of therapy. The most important significant associations with a cardiotoxic reaction were with hemoglobin and NT-proBNP. Interestingly and unexpectedly, hsTrop T was

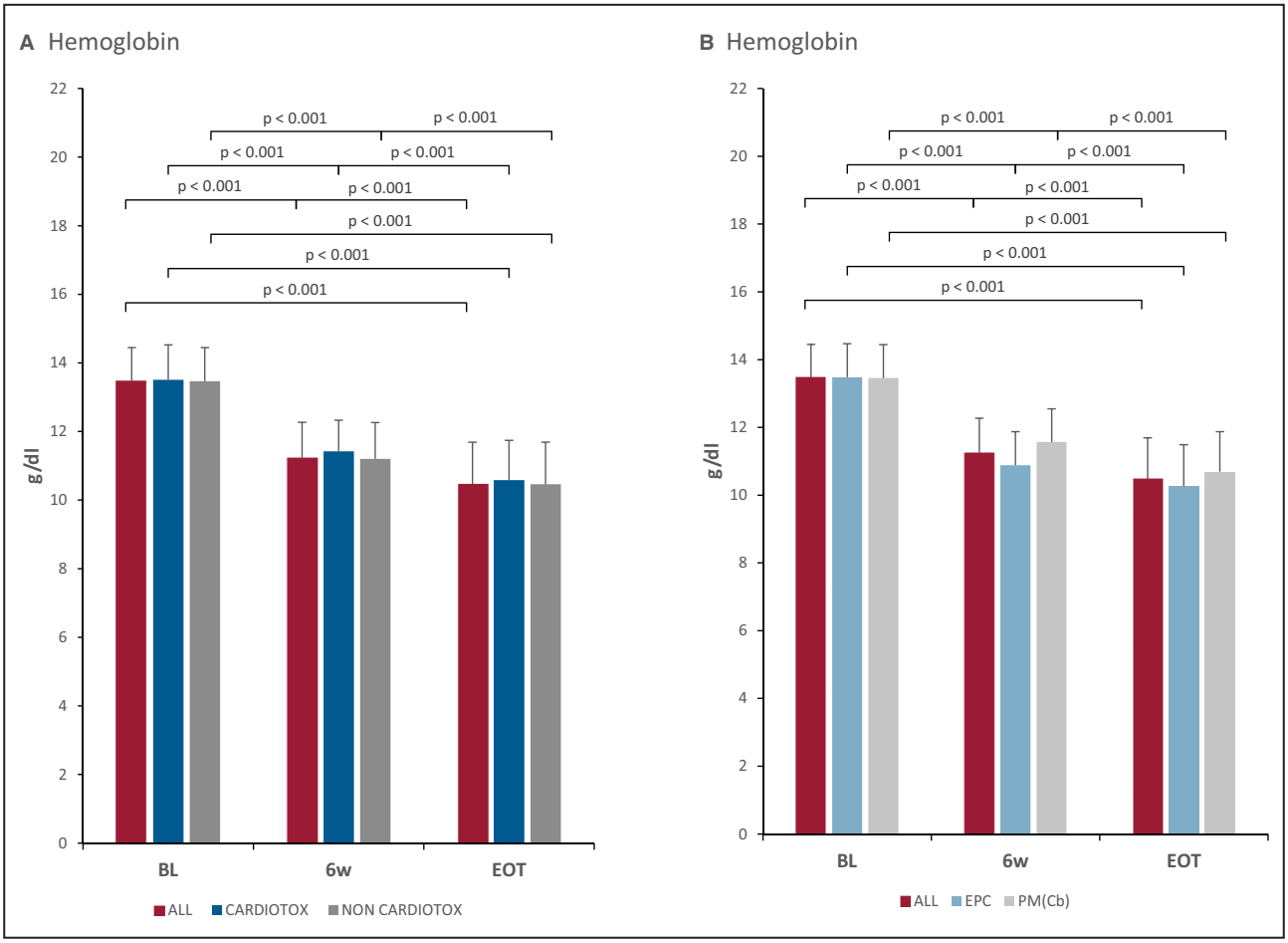

Figure 5. Hemoglobin during therapy.
A, Hemoglobin (cardiotoxicity vs non-cardiotoxicity); (**B**) Hemoglobin (neo-adjuvant dose-dense, dose-intensified epirubicin, paclitaxel, and cyclophosphamide vs paclitaxel, non-pegylated doxorubicin, carboplatin). Hemoglobin as assessed by all (red), cardiotoxicity (blue bar) vs non-cardiotoxicity (grey bar) and between all (red), EPC (light blue bar) and PM(Cb) (light grey bar) group. Analysis is shown as mean±SD and independent samples t-test shown by *P* values. The time points of assessment are baseline, after 6 weeks, and at the end of therapy. iddEPC indicates neo-adjuvant dose-dense, dose-intensified epirubicin, paclitaxel, and cyclophosphamide; and PM(Cb), paclitaxel, non-pegylated doxorubicin, carboplatin.

not significantly associated with cardiotoxic reactions. This remained true even though there were significant differences between hsTrop T at baseline and at the end of therapy for both definitions of cardiotoxicity used. Treatment allocation in the trial and HER2 receptor status, the latter determining the use of HER2 antibody therapy, did not predict cardiotoxic reactions, implying that the main driver of this complication was determined by the underlying anthracycline therapy.

Few studies have evaluated the role of cardiac biomarkers in patients undergoing chemotherapy for breast cancer even though cardiotoxic chemotherapies belong to the most relevant treatment strategies in this patient group. Cardiac stress biomarkers are viewed as potentially valid cardiotoxicity predictors, and the few available studies have particularly focused on the troponins and NT-proBNP.[19,20] Zardavas et al, for example, evaluated the role of troponin I, troponin T, and NT-proBNP among 452 patients with early breast cancer from the HERA (Herceptin Adjuvant) trial. These authors used the cardiac troponin I (cTnI) Ultra assay for troponin I assessment in which a value >40 ng/L marks an increase >99th percentile of a healthy population; 13.6% of all baseline values were elevated. Indeed, troponin I is a marker of myocyte necrosis, and its rise may indicate the magnitude and time course of cell injury.[21] Cardinale et al have shown that a troponin I rise within 3 days after high-dose chemotherapy predicts reduction in LVEF.[22] Zardavas et al used the same test like we did, the high-sensitivity cardiac troponin T (cTnT) test with a value >14 ng/L representing the 99th percentile. Whilst in our analysis, only 0.35% of all patients had elevated values at baseline, the corresponding value in the analysis by Zardavas et al was an astonishing 24.8%. In contrast to our results, Zardavas et

Table 2. Univariate and Multivariate Logistic Regression Model to Predict Cardiotoxicity in Patients With Breast Cancer

Variable	Univariate Model*			Multivariate Model†		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Age (per y)	1.01	0.99–1.03	0.35	1.01	0.99–1.03	0.39
BMI (per kg/m^2)	1.00	0.96–1.04	0.98	0.99	0.95–1.04	0.74
Arm (iddEPC vs PM(Cb))	1.22	0.83–1.81	0.31	0.99	0.60–1.63	0.95
Creatinine (per mg/dL)	3.74	0.89–15.60	0.07	3.36	0.74–15.3	0.12
Leukocytes (per/nL)	1.00	0.91–1.09	0.93	0.96	0.87–1.07	0.49
LVEF (per %)	1.26	1.21–1.32	<0.001			
Diabetes mellitus (present)	1.09	0.32–3.78	0.89	0.90	0.23–3.53	0.88
Dyslipidemia (present)	1.67	0.54–5.11	0.37	1.69	0.52–5.52	0.39
Hypertension (present)	0.76	0.43–1.33	0.33	0.70	0.37–1.31	0.26
Blood pressure (systolic) (per mm Hg)	1.00	0.98–1.02	0.94			
Pulse (per/min)	1.00	0.98–1.02	0.95			
HER2 (present)	0.99	0.66–1.46	0.94			
NT-proBNP (per 10 pg/mL)	1.00	0.97–1.03	0.91			
hsTrop T (per ng/L)	1.02	0.90–1.16	0.76			
Hemoglobin (per g/dL), 6 w	1.23	1.02–1.49	0.03	1.31	1.05–1.63	0.02
LVEF (per %), 6 w	0.95	0.91–0.98	0.002			
NT-proBNP (per 10 pg/mL), 6 w	1.02	1.00–1.04	0.07	1.03	1.01–1.06	0.01
Leukocytes (per/nL), 6 w	1.02	0.97–1.06	0.52			
hsTrop T (per ng/L), 6 w	1.00	0.96–1.04	0.83	0.97	0.91–1.03	0.28
Pulse under therapy, 6 w	1.01	0.99–1.03	0.30			
Blood pressure (systolic) under therapy (per mm Hg), 6 w	1.01	0.99–1.02	0.49			
Platelets (per 10/nL), 6 w	1.00	0.98–1.03	0.91			

Values are odds ratio (95% CI). Multivariate model is adjusted for age, BMI, arm, creatinine, leukocytes, diabetes mellitus, dyslipidemia, hypertension, hemoglobin at 6 weeks, NT-proBNP at 6 weeks, hsTrop T at 6 weeks. BMI indicates body mass index; HER2, human epidermal growth factor receptor 2; hsTrop T, high-sensitivity cardiac troponin T; iddEPC, neo-adjuvant dose-dense, dose-intensified epirubicin, paclitaxel, and cyclophosphamide; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; OR, odds ratio; PM(Cb), paclitaxel, non-pegylated doxorubicin, carboplatin.

P values from univariate* and multivariate† logistic regression models. Dependent variable is cardiotoxicity.

al found that both baseline troponin tests predicted a significant drop in LVEF.[21] Like in our analysis, these authors found higher increases in NT-proBNP from baseline to be associated with an increased risk of a significant LVEF drop. Ponde et al used data from 280 patients with early breast cancer treated in the NeoALTTO trial to assess the predictive power of troponin T and NT-proBNP using the same assays as we did.[23] In their analysis, elevations in biomarker levels were rare and failed to find an association between these markers and cardiac events.

NT-proBNP is an indicator of cardiac strain and/or volume overload but might not show early cell injury, as is the case with the troponins.[12] However, previous studies have supported the predictive value of NT-proBNP. Biological variation and variability with increasing age, body mass index, and worsening renal function may require attention as well when looking at the biomarker results.[24,25] Unfortunately, many earlier studies were hampered by sample size, timing, and validity of cardiac assessments, or both. Auner et al, for example, have shown that cardiac troponin T is eligible for early prediction of cardiotoxic reactions in patients with cancer induced via anthracycline therapy. The study in 78 patients with hematological malignancy has shown that even short-term and minor anthracycline therapy has effects on troponin T levels.[26] Adamson et al conducted a recent breast cancer study to assess the role of biomarkers in predicting cardiotoxic reactions. Again, troponin elevations were used to show myocardial injury already at the beginning of breast cancer therapy.[27] Kitayama et al conducted a study on patients with breast cancer and showed that 4 of 40 patients with cardiotoxic reaction were treated with epirubicin and trastuzumab, and that these patients had higher values of hsTrop T.[28] Few studies have used NT-proBNP as a marker for predicting future cardiotoxic events. Sandri et al studied the role of NT-proBNP among 52 patients with aggressive malignancies. Echocardiography was conducted out to 72 hours after high-dose chemotherapy, and a 1-year follow-up was done. Importantly, patients with increased NT-proBNP levels had a significant decrease in LVEF from baseline to 12 months. Therefore, early rises of NT-proBNP

levels seem to be indeed correlated with cardiotoxic reactions.[29]

Similar findings were published by Feola et al already in 2011. They recruited patients who had undergone surgical resection and who were eligible for adjuvant chemotherapy. Clinical assessments, radionuclide ventriculography, troponin I, and B-type natriuretic peptide (BNP) assessments were scheduled at 1 month, 1 year, and 2 years of follow-up. Reductions in LVEF ≥10% or overt heart failure were the events of interest. BNP showed minor but statistically significant increases from 33.4±41.5 to 62.7±94.7 pg/mL after 1 year. Troponin I displayed significant increases within the first month and then normalized again during the overall study duration. Only baseline BNP levels showed a relevant correlation with LVEF after 2 years. With 32% of all patients showing a reduction in LVEF, this proportion was much higher than in our study, but the treatment also differed. However, patients with a cardiotoxic reaction had higher baseline BNP and lower hemoglobin levels than the patient group without cardiotoxic reactions.[25] This coincides with our findings on NT-proBNP, but especially also with our findings on hemoglobin levels.

The definition of cardiotoxicity varies across studies and an international consensus has not been achieved as yet and remains a matter of debate. Prevalence values varied in our study between 12.9% and 1.8%, depending on the definition used. Longer follow-up periods are welcome. For example, the committee of the cardiac review and evaluation supervising trastuzumab clinical trials defined cardiotoxicity induced by chemotherapy as ≥1 of the following criteria: (1) reduction in LVEF, interventricular specific or global, (2) signs or symptoms associated with heart failure, and (3) LVEF reduction from baseline up to ≤5% to <55% with symptoms or signs of heart failure or LVEF reduction from ≥10% to <55% without heart failure signs or symptoms.[30,31] Other workers have used different approaches and definitions. For example, Cardinale et al assessed the importance of troponin as a predictor of cardiotoxicity using a reduction in LVEF ≥10 percentage points over time in various studies[19,20,22] just like we did. Ky et al defined cardiotoxicity as a reduction in LVEF ≥5% up to <55% with symptoms of heart failure or asymptomatic reduction in LVEF ≥10% up to <55%.[32] Wehner et al have recently shown that LVEF values are correlated in a U-shape fashion with mortality rates with a nadir at 60% to 65%, implying that even high values are not beneficial.[33]

The difference in the reversibility of the cardiotoxic reactions lies in the mechanisms of treatment. Trastuzumab inhibits HER2 mediated mechanisms to protect cardiac myocytes under stress. Anthracycline-induced cardiotoxicity, which may become detectable early, arises from oxidative stress, associated with reactive oxygen species within cardiomyocytes. The major mediator herein is topoisomerase 2ß, whose inhibition can lead to DNA breaks with consecutive cardiomyocyte death.[27,34,35] The combination of both therapy options, anthracycline therapy and trastuzumab, leads to a 7-times higher risk of heart failure or cardiomyopathy,[35,36] but the time of onset may be well outside the observation time of standard studies. Lifestyle factors like smoking, obesity, high alcohol intake, and sedentary lifestyle should be taken into consideration. These often interact with breast cancer risk factors like age, diet, tobacco use, obesity, and sedentary lifestyle.[1,37] As outlined, the incidence of cardiotoxicity depends on the choice of chemotherapy, underlying comorbidities, therapy duration, and predisposing demographic risk factors. These reasons lead to additive or even synergetic effects, which trigger cardiotoxicity.[13,38,39] Our data buttress the view that NT-proBNP is a useful marker in this regard, but studies with longer follow-up are needed to better understand the course of LVEF reduction over time and the development of manifest heart failure. Given the above study data, the assessment of a biomarker portfolio at therapy initiation including high-sensitivity troponin and NT-proBNP appears to be reasonable. The significance of a biomarker-based approach to screening of cardiotoxicity gains even more relevance within a pandemic, as Coronavirus disease 2019, where exposure times for echocardiograms are not ideal.

ARTICLE INFORMATION

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