ESC HEART FAILURE
ESC Heart Failure 2022; **9**: 1127–1137
Published online 2 February 2022 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.13782

ORIGINAL ARTICLE

Early detection of anthracycline- and trastuzumab-induced cardiotoxicity: value and optimal timing of serum biomarkers and echocardiographic parameters

Belén Díaz-Antón[1,2,3,4], Rodrigo Madurga[5,6], Blanca Zorita[1], Samantha Wasniewski[2], Andrea Moreno-Arciniegas[1,2], Beatriz López-Melgar[1,2], Natalia Ramírez Merino[3,7], Roberto Martín-Asenjo[8], Patricia Barrio[1,2], Maximiliano German Amado Escañuela[2,9], Jorge Solís[4,8,10,12], Francisco Javier Parra Jiménez[1], Eva Ciruelos[7,11], José María Castellano[1,3,4,5,10,12] and Leticia Fernández-Friera[2,3,4,10,12]*

[1]*Departamento de Cardiología, HM Hospitales-Centro Integral de Enfermedades Cardiovasculares HM CIEC, Melchor Fernández Almagro 3, Madrid, 28029, Spain;* [2]*Unidad de Imagen Cardiaca, HM Hospitales-Centro Integral de Enfermedades Cardiovasculares HM CIEC, Madrid, Spain;* [3]*Universidad CEU San Pablo, Madrid, Spain;* [4]*Atria Clinic, Madrid, Spain;* [5]*Fundación de Investigación HM Hospitales, HM Hospitales, Madrid, Spain;* [6]*Facultad de Ciencias Experimentales, Universidad Francisco de Vitoria, Madrid, Spain;* [7]*Departamento de Oncología Médica, HM Hospitales, Madrid, Spain;* [8]*Servicio de Cardiología, Hospital Universitario 12 de Octubre, Madrid, Spain;* [9]*Hospital General de Segovia, Segovia, Spain;* [10]*Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain;* [11]*Servicio Oncología, Hospital Universitario 12 de Octubre, Madrid, Spain; and* [12]*CIBER de enfermedades CardioVasculares (CIBERCV), Madrid, Spain*

Abstract

Aims To evaluate echocardiographic and biomarker changes during chemotherapy, assess their ability to early detect and predict cardiotoxicity and to define the best time for their evaluation.

Methods and results Seventy-two women with breast cancer (52 ± 9.8 years) treated with anthracyclines (26 also with trastuzumab), were evaluated for 14 months (6 echocardiograms/12 laboratory tests). We analysed: high-sensitivity cardiac troponin T, NT-proBNP, global longitudinal strain (GLS), left ventricle end-systolic volume (LVESV), left ventricle end-diastolic volume (LVEDV), and left ventricular ejection fraction (LVEF). Cardiotoxicity was defined as a reduction in LVEF>10% compared with baseline with LVEF<53%. High-sensitivity troponin T levels rose gradually reaching a maximum peak at 96 ± 13 days after starting chemotherapy ($P < 0.001$) and 62.5% of patients presented increased values during treatment. NT-proBNP augmented after each anthracycline cycle (mean pre-cycle levels of 72 ± 68 pg/mL and post-cycle levels of 260 ± 187 pg/mL; $P < 0.0001$). Cardiotoxicity was detected in 9.7% of patients (mean onset at 5.2 months). In the group with cardiotoxicity, the LVESV was higher compared with those without cardiotoxicity (40 mL vs. 29.5 mL; $P = 0.045$) at 1 month post-anthracycline treatment and the decline in GLS was more pronounced (−17.6% vs. −21.4%; $P = 0.03$). Trastuzumab did not alter serum biomarkers, but it was associated with an increase in LVESV and LVEDV ($P < 0.05$). While baseline LVEF was an independent predictor of later cardiotoxicity ($P = 0.039$), LVESV and GLS resulted to be early detectors of cardiotoxicity [odds ratio = 1.12 (1.02–1.24), odds ratio = 0.66 (0.44–0.92), $P < 0.05$] at 1 month post-anthracycline treatment. Neither high-sensitivity troponin T nor NT-proBNP was capable of predicting subsequent cardiotoxicity.

Conclusions One month after completion of anthracycline treatment is the optimal time to detect cardiotoxicity by means of imaging parameters (LVESV and GSL) and to determine maximal troponin rise. Baseline LVEF was a predictor of later cardiotoxicity. Trastuzumab therapy does not affect troponin values hence imaging techniques are recommended to detect trastuzumab-induced cardiotoxicity.

Keywords Cardiotoxicity; Anthracyclines; Trastuzumab; High-sensitivity cardiac troponin; Global longitudinal strain

Received: 1 May 2021; Revised: 13 September 2021; Accepted: 14 December 2021
**Correspondence to: Leticia Fernández-Friera, Departamento de Cardiología, HM Hospitales, Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Melchor Fernández Almagro 3, 28029 Madrid, Spain. Email: lafernandez@cnic.es*
José María Castellano and Leticia Fernández-Friera contributed equally to the manuscript.

© 2022 The Authors. ESC Heart Failure published by John Wiley & Sons Ltd on behalf of European Society of Cardiology.
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Introduction

Anthracyclines and trastuzumab are first-line antitumoural agents used in breast cancer therapy.[1] However, they can induce cardiotoxicity, especially in patients with a high cardiovascular risk, a background of mediastinal radiotherapy, or concomitant therapy with other agents such as cyclophosphamide.[2,3] Cardiotoxicity can appear with any treatment dose and also in the absence of the aforementioned factors, so it is a challenge to detect. Early detection of myocardial injury is key to reducing cardiovascular complications and to avoid having to discontinue antitumoural therapy.[4]

Given that classical biomarkers such as troponin I and T and global longitudinal strain (GLS) have been described as predictors of cardiotoxicity,[5–7] these should be monitored during treatment with cardiotoxic agents.[8] NT-proBNP is of doubtful value as a predictor of cardiotoxicity, and previously published results are contradictory.[9] High-sensitivity troponin levels in this setting are even less validated, and some studies have described elevated values in 28% of patients.[10] However, specific threshold values for cardiotoxicity and the optimum timing for extractions are still unknown. On the other hand, myocardial deformation techniques are robust and reproducible, although dependent on image quality, with wide variability among results from different ultrasound equipment and no clear evidence about the best time to perform them.[11,12]

The main objectives of this work were to assess changes in echocardiographic parameters [left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), and GLS] and in biomarkers [high-sensitivity cardiac troponin T (hs cTnT) and NT-proBNP] extracted during treatment with anthracyclines, with or without trastuzumab, and to assess their capacity to early detect and predict cardiotoxicity. Other objectives included (i) determining the best time to evaluate echocardiographic parameters and biomarkers; (ii) assessing the toxicity pattern of different chemotherapy regimens (anthracyclines vs. anthracyclines with trastuzumab).

Methods

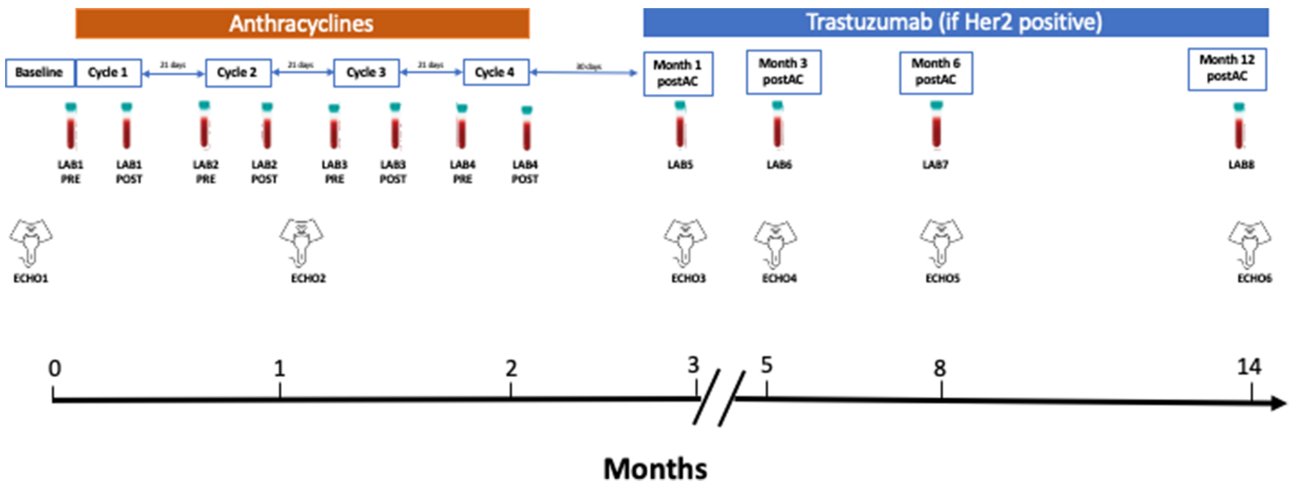
Study population

Patients over 18 years old with non-metastatic breast cancer referred to our centre between January 2017 and August 2018 and considered as candidates for anthracycline treatment were prospectively and consecutively included. The presence of cardiovascular risk factors, previous cardiac diseases, renal, or hepatic dysfunction were not exclusion factors. The chemotherapy regimen is described in *Figure S1*. Trastuzumab was administered a minimum of 1 month after completing anthracycline treatment. The study was approved by the hospital ethics research committee (CEIm HM hospitales:16.11.1014-GHM) and all patients signed the informed consent.

Study protocol

The study protocol is shown in *Figure 1*. The study duration was 14 months and included 6 echocardiography evaluations and 12 blood tests. High-sensitivity troponin T and NT-proBNP were measured before and after each anthracycline cycle, with a 24–48 h margin (LAB1-LAB4). After

Figure 1 Diagram showing the study protocol. AC, anthracyclines; ECHO, echocardiogram; LAB, laboratory (Troponin and NT-proBNP); POST, post-cycle; PRE, pre-cycle.



DOI: 10.1002/ehf2.13782

completing anthracycline treatment, patients were monitored again at the end of 1, 3, 6, and 12 months (LAB5-LAB8). Echocardiography was performed before starting chemotherapy (ECHO1) and was repeated between the second and third cycles of anthracycline therapy (ECHO2), and also after completing all cycles at the end of 1, 3, 6, and 12 months (ECHO3-ECHO6). Cardiotoxicity was defined as a reduction in LVEF >10% compared with baseline value with an LVEF <53%, in accordance with the most widely used definition established in the consensus document by Plana *et al*.[8] At the moment of cardiotoxicity detection on the echocardiogram, cardioprotective therapies with beta-blockers and ACE-inhibitors/sartans were initiated and maintained throughout the 14 month follow-up.

Transthoracic echocardiogram

All studies were acquired with a Philips Epiq 7 ultrasound system (Philips Health Care, Andover, MA, USA) by a cardiologist qualified in imagining techniques accredited by the European Association of Cardiovascular Imaging. Measurements were taken off-line in the Xcelera workstation. Echocardiographic parameters included LVEDV, LVESV, and LVEF, following recommendations for the quantification of cardiac chambers.[13] GLS was quantified using QLAB 10.5 (Philips Health Care, Andover, MA, USA) and Automated Cardiac Motion Quantification software (aCMQ). Borders of the endocardium were manually traced in telediastole in three-chamber, four-chamber, and two-chamber views, and strain curves were obtained automatically.[13]

Biomarkers

Serum hs cTnT levels were measured with the Elecsys of Troponin T hs assay (Roche). The lower detection limit was 3 ng/L (defined as undetectable levels) and levels >14 ng/L (99th percentile of healthy individuals) were considered elevated.

Serum NT-proBNP levels were measured with the Elecsys of proBNP II assay (Roche) and values >125 pg/ml (99th percentile of healthy individuals) were considered elevated.

Statistical analysis

Quantitative variables are presented as means ± standard deviations and categorical variables as absolute and relative frequencies (%). Differences between patients with and without cardiotoxicity were determined by the Student-t test for quantitative variables and χ^2 test for categorical variables, recurring to Wilcoxon or Fischer's test in non-parametric conditions. In cases for which multiple tests were applied, *P* values were adjusted by the Benjamin–Hochberg method. Predictive capacity was determined by univariate logistic regression analysis. For predictive variables in the univariable model ($P < 0.05$), the receiver-operating characteristic (ROC) curve was computed and the cut-off that produced maximum sensitivity and specificity was considered. Intra-observer and inter-observer agreement were calculated from the difference in values obtained in 20 patients using the intraclass correlation coefficient (ICC) and the Bland–Altman analysis. Statistical analysis was performed using R version 4.0.0. An α limit of 0.05 was considered as statistically significant.

Results

Eighty patients were included in the study and 90% of cases (n = 72) completed the 14 month follow-up. Out of the eight patients that did not complete the study protocol, three discontinued chemotherapy treatment early due to non-cardiological toxicity and five patients changed institution for follow-up. Baseline characteristics of the cohort are summarized in *Table 1*, broken down by cardiotoxicity. All study participants (100%) were women (mean age 52.0 ± 9.8 years) and 5 presented a cardiac disease (1 mechanical mitral valve prosthesis, 1 left bundle branch block, 2 hypertensive cardiopathy, and 1 mitral insufficiency). All patients (100%) received anthracyclines (epirubicin, 88.9%), in 36.1% cases combined with trastuzumab, and 97.2% received additional thoracic radiotherapy.

A total of 9.7% developed cardiotoxicity (reduction of LVEF >10% vs. baseline value with LVEF <53%) with a mean onset at 5.2 ± 1.3 months (5 patients developed it 3 months post-anthracycline treatment, 1 patient one-month post-anthracyclines and 1 patient 6 months post-anthracyclines). Patients that developed cardiotoxicity were slightly younger (45.7 ± 7.3 years) and free of cardiovascular risk factors. Baseline levels of LVEF, GLS, and NT-proBNP were no different between the groups with or without cardiotoxicity, whereas hs cTnT was slightly raised in the group that did not develop cardiotoxicity (4.8 ± 2.1 vs. 3.1 ± 0.2; P = 0.006), but without reaching pathological levels (>14 ng/L) in any patient. Among patients with cardiotoxicity, one presented dyspnoea and the rest were asymptomatic but all received beta-blockers and ACE-inhibitors or sartans at maximum tolerated doses. One patient required temporary interruption of trastuzumab. During follow-up, all patients that developed cardiotoxicity normalized LVEF and strain values.

Changes in echocardiographic parameters and biomarkers

Echocardiographic parameters at baseline and during follow-up of the whole cohort and broken down by presence

Table 1 Baseline characteristics of study cohort

	Total (n = 72)	No cardiotoxicity (n = 65)	Cardiotoxicity (n = 7)	P value
Age (years)	52.0 ± 9.8	52.7 ± 9.8	45.7 ± 7.3	0.08
Cardiovascular risk factors				
Active smoking	4 (5.6%)	4 (6.2%)	0 (0.0%)	1
Diabetes mellitus	1 (1.4%)	1 (1.5%)	0 (0.0%)	1
Dyslipidaemia	6 (8.3%)	6 (9.2%)	0 (0.0%)	1
Arterial hypertension	5 (6.9%)	5 (7.7%)	0 (0.0%)	1
Body mass index	24.8 ± 3.9	25.0 ± 4.0	22.5 ± 1.7	0.53
Baseline creatinine (mg/dL)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.87
Heart rate (bpm)	69.0 ± 12.4	69.1 ± 12.8	67.7 ± 7.7	0.91
Systolic BP (mmHg)	107.7 ± 16.8	107.7 ± 17.4	107.1 ± 9.5	0.87
Diastolic BP (mmHg)	67.7 ± 9.5	67.9 ± 9.9	65.7 ± 5.3	0.49
Oncological variables				
Anthracyclines				1
Epirubicin	64 (88.9%)	57 (87.7%)	7 (100%)	
Doxorubicin	8 (11.1%)	8 (12.3%)	0 (0.0%)	
Anthracycline dose (mg/m^2)				1
Epirubicin	360	360	360	
Doxorubicin	200	200	200	
Radiotherapy	70 (97.2%)	63 (96.9%)	7 (100%)	1
Trastuzumab	26 (36.1%)	22 (33.8%)	4 (57.1%)	0.24
Echocardiographic parameters				
LVEDV (mL)	81.3 ± 16.3	81.0 ± 16.6	84.0 ± 15.0	0.5
LVESV (mL)	30.0 ± 8.2	29.5 ± 8.0	34.4 ± 9.7	0.19
LVEF (%)	63.3 ± 5.4	63.8 ± 5.2	59.4 ± 6.5	0.07
GLS (%)	−21.8 ± 2.3	−21.9 ± 2.3	−20.9 ± 2.2	0.21
Biomarkers				
Hs cTnT (ng/L)	4.6 ± 2.0	4.8 ± 2.1	3.1 ± 0.2	0.006
NT-ProBNP (pg/mL)	82.3 ± 95.3	84.3 ± 99.9	65.4 ± 39.4	0.94

GLS, global longitudinal strain; Hs cTnT, high-sensitivity troponin T; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume.

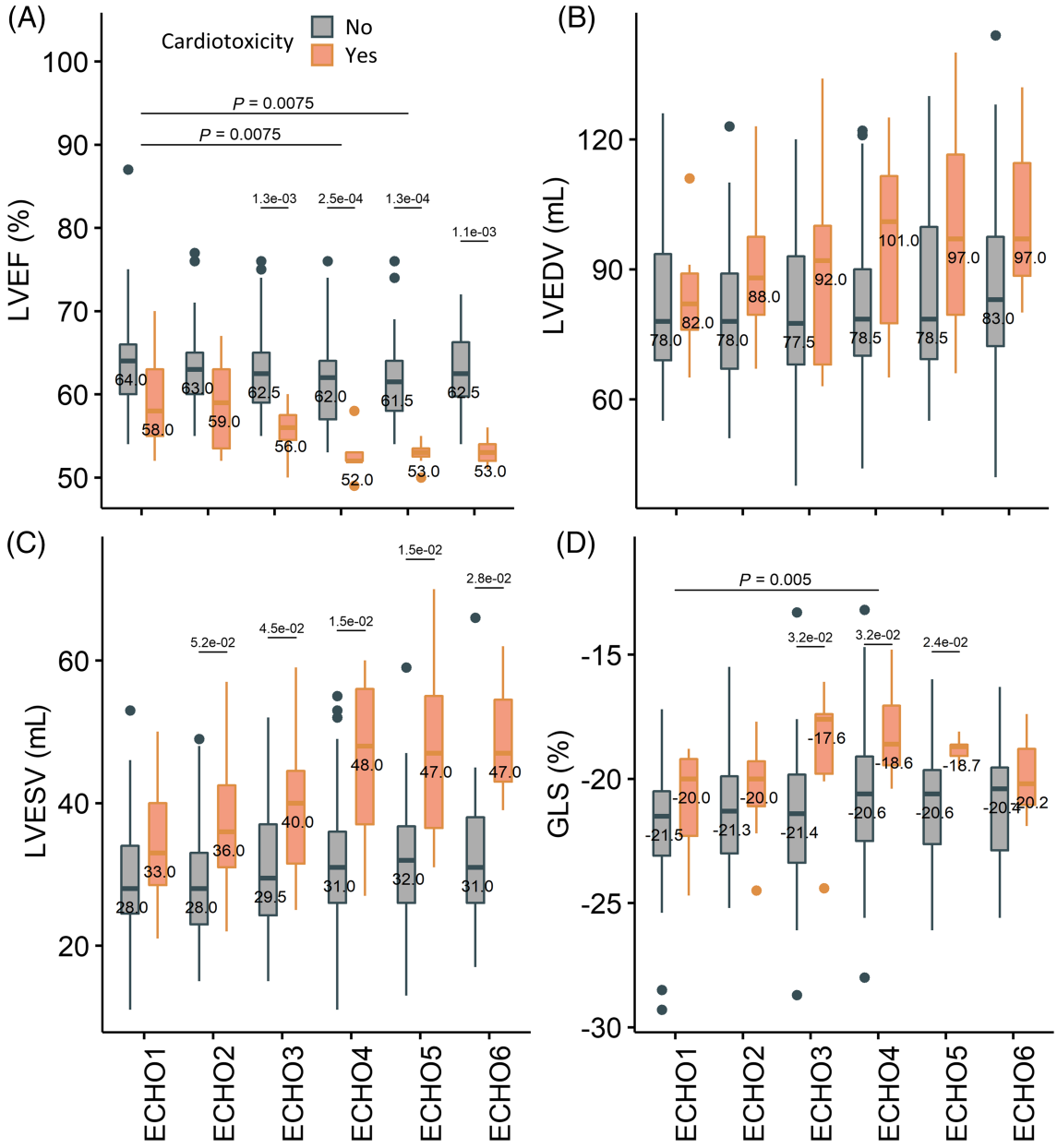
of cardiotoxicity are shown in *Figures S2 and 2*. Globally, for the whole cohort, LVEF decreased significantly after ECHO4. This decline in LVEF occurred earlier in the group that developed cardiotoxicity (ECHO3) than in the group without cardiotoxicity reaching criteria of cardiotoxicity (reduction in LVEF >10% vs. a baseline value with LVEF <53%) in ECHO4 (LVEF = 52.7% ± 2.7% vs. 61.4% ± 4.8%, $P < 0.001$). The LVEDV tended to rise in the cardiotoxicity group, although differences between groups were not significant. The LVESV also showed a tendency to increase in the group with cardiotoxicity from 34.4 ± 9.7 mL in ECHO1 to 49.3 ± 11.7 in ECHO6, and reached significant differences compared with the group without cardiotoxicity in ECHO3 (P = 0.045). Globally, in the whole cohort, GLS was significantly reduced in ECHO4. In the group presenting cardiotoxicity, the decline in GLS was more pronounced and occurred earlier, reaching values in ECHO3 (1 month after completing anthracycline treatment) of −18.9% ± 2.8% compared with baseline values of 20.9% ± 2.2% (P = 0.032), reaching also significant differences between the groups at this time (P = 0.03).

Regarding the hs cTnT levels, neither of the groups presented elevated values at baseline (>14 ng/L) and in 36.7% of patients these values were undetectable (<3 ng/L). The hs cTnT rose steadily and significantly with consecutive anthracycline cycles, from 4.6 ± 2.1 ng/L in the baseline blood test to a maximum value of 15.7 ± 7.4 ng/L at 96 ± 13 days (*Figure 3*). At 12 months after completing anthracycline treatment, levels had fallen to 5.9 ± 2.1 ng/L. Over the treatment period, 62.5% of patients presented pathological values (>14 ng/L). Levels of hs cTnT were found to be linearly correlated with age ($P < 0.001$, $R^2 = 0.16$) and baseline levels as well as levels during follow-up were significantly higher in women >55 years than in women ≤55 years old (*Figure S3*). Differences were not significant between hs cTnT results extracted before vs. after the anthracycline cycles (*Figure 4A*), or between groups with or without cardiotoxicity, either at baseline or during follow-up (*Table 2*). Baseline NT-proBNP was normal in all patients and was not age dependent. Values of NT-proBNP extracted before and after anthracycline cycles were significantly different ($P < 0.0001$), with mean pre-cycle levels of 72 ± 68 pg/mL and post-cycle levels of 260 ± 187 pg/mL (*Figure 4B*). NT-proBNP levels were not significantly different between patient groups with and without cardiotoxicity (*Table 2*).

Effects of trastuzumab

Table S1 shows clinical variables, echocardiographic parameters, and biomarker levels at baseline and during follow-up in patients with and without trastuzumab. No significant differences were observed between groups regarding clinical variables or biomarkers. For echocardiographic parameters, a

Figure 2 Echocardiographic parameters at baseline and during follow-up broken down by presence of cardiotoxicity (values expressed as median and interquartile range).ECHO1, baseline; ECHO2, 2nd–3rd anthracycline cycle; ECHO3, 1st month post-anthracyclines; ECHO4, 3rd month post-anthracyclines; ECO5, 6th month post-anthracyclines; ECO6, 12th month post-anthracyclines; GLS, global longitudinal strain; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume.



significant rise in LVEDV and LVESV was observed but no significant differences in LVEF or GLS.

Predictive value of cardiotoxicity for echocardiographic parameters and biomarkers

The results for baseline variables and those determined at 1 month post-anthracycline treatment are recorded in *Table 3*. At baseline study, LVEF was the only variable that could predict later cardiotoxicity ($P = 0.039$). However, after completing anthracycline therapy LVEF, LVESV and GLS turned out to be early detectors of cardiotoxicity [odds ratio (OR) = 0.60 (0.37–0.81), OR = 1.12 (1.02–1.24), OR = 0.66 (0.44–0.92), $P < 0.05$]. Neither the decline in LVEF vs. baseline values (% drop) nor the biomarkers (hs cTnT and NT-proBNP) were capable of predicting subsequent cardiotoxicity. After analysing the ROC curve for these

DOI: 10.1002/ehf2.13782

Figure 3 Evolution of high-sensitivity troponin T. LAB1, 1st anthracycline cycle; LAB2, 2nd anthracycline cycle; LAB3, 3rd anthracycline cycle; LAB4, 4th anthracycline cycle; LAB5, 1st month post-anthracyclines; LAB6, 3rd month post-anthracyclines; LAB7, 6th month post-anthracyclines; LAB8, 12th month post-anthracyclines.

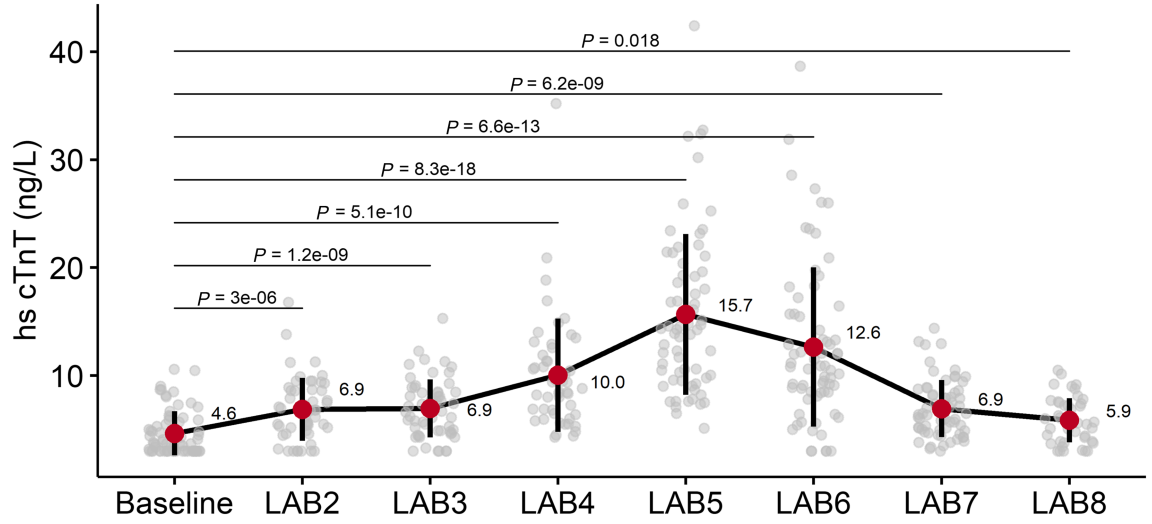
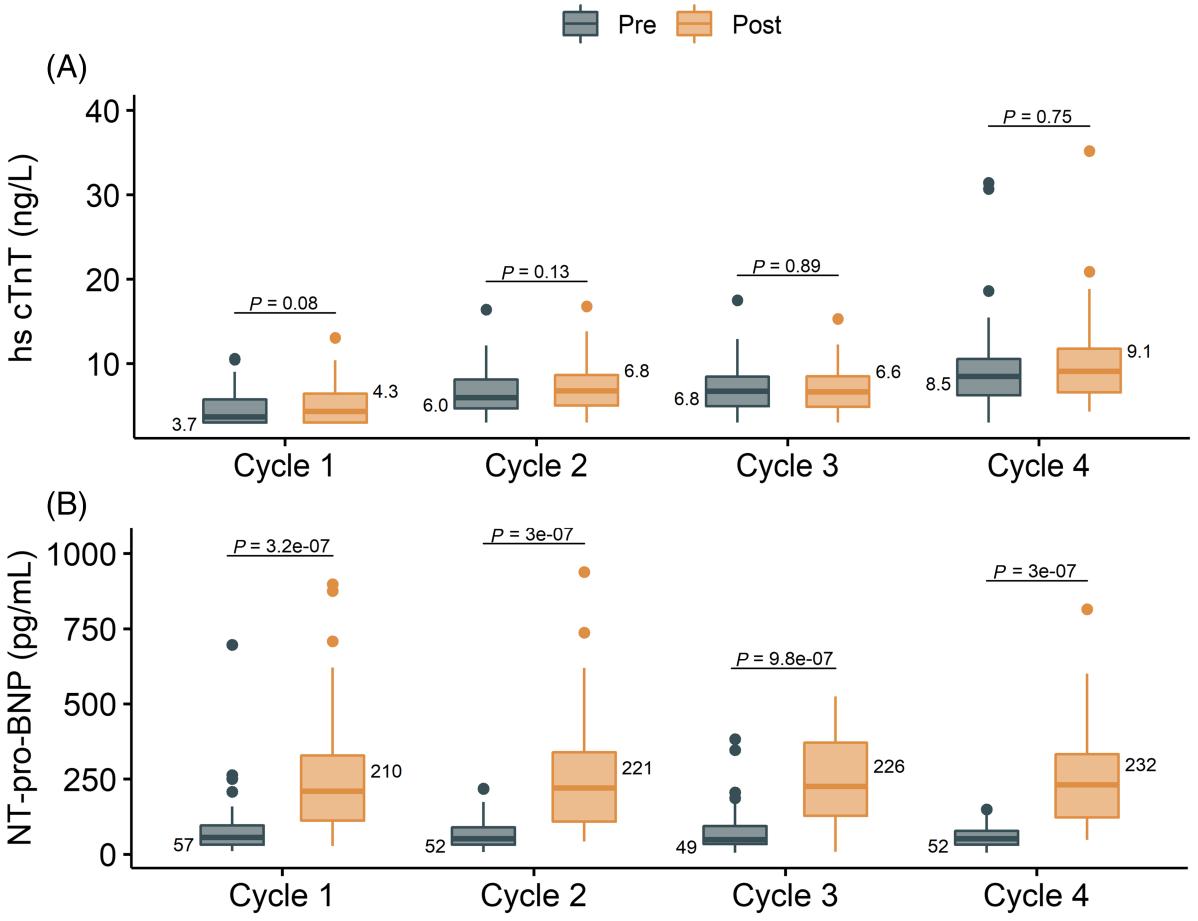


Figure 4 (A) Troponin values before and after the anthracycline cycles; (B) NT-proBNP values before and after the anthracycline cycles.



DOI: 10.1002/ehf2.13782

Table 2 Biomarker concentrations over the course of visits broken down by cardiotoxicity

		LAB1	LAB2	LAB3	LAB4	LAB5	LAB6	LAB7	LAB8
No cardiotoxicity (n = 65)	NT-proBNP (pg/mL)	84.3 ± 99.9	60.6 ± 44.2	76.0 ± 70.1	60.3 ± 36.2	76.3 ± 66.8	65.1 ± 63.2	66.4 ± 55.3	75.0 ± 53.1
	Hs cTnT (ng/L)	4.8 ± 2.1*	6.6 ± 2.6	7.3 ± 2.9	9.5 ± 5.1	16.0 ± 7.6	12.7 ± 7.5	7.1 ± 2.6**	5.9 ± 2.1
Cardiotoxicity (n = 7)	NT-proBNP (pg/mL)	65.4 ± 39.4	96.8 ± 65.7	97.3 ± 69.6	69.0 ± 47.3	73.1 ± 58.6	47.1 ± 22.3	93.9 ± 69.0	82.5 ± 75.9
	Hs cTnT (ng/L)	3.1 ± 0.2*	5.1 ± 0.7	5.0 ± 0.9	6.4 ± 2.0	12.7 ± 4.7	11.8 ± 6.9	4.4 ± 1.4**	4.2 ± 0.4

Hs cTnT, high-sensitivity troponin T; LAB1, 1st anthracycline cycle; LAB2, 2nd anthracycline cycle; LAB3, 3rd anthracycline cycle; LAB4, 4th anthracycline cycle; LAB5, 1st month post-anthracyclines; LAB6, 3rd month post-anthracyclines; LAB7, 6th month post-anthracyclines; LAB8, 12th month post-anthracyclines.
*Adjusted $P < 0.05$.
**Adjusted $P < 0.1$.

Table 3 Univariate analysis of cardiotoxicity predictors

Variable	*P* value	Odds ratio	Confidence interval (95%)
Baseline variables			
Age (years)	0.079	0.921	0.833–1.004
Trastuzumab	0.24	2.606	0.530–14.223
LVEF (%)	0.039	0.810	0.647–0.974
LVESV (mL)	0.14	1.071	0.977–1.179
LVEDV (mL)	0.64	1.011	0.962–1.059
GLS (%)	0.3	1.228	0.855–1.881
NT pro-BNP (pg/mL)	0.62	0.997	0.978–1.005
Hs cTnT (ng/L)	0.13	0.066	0.0002–0.567
At 1 month post-anthracyclines			
LVEF (%)	0.007	0.601	0.370–0.807
LVESV (mL)	0.018	1.117	1.024–1.238
LVEDV (mL)	0.28	1.022	0.981–1.066
GLS (%)	0.024	0.662	0.438–0.919
NT pro-BNP (pg/mL)	0.9	0.999	0.985–1.010
Hs cTnT (ng/L)	0.27	0.923	0.780–1.042
Changes between 1 month post-anthracyclines and baseline values			
Reduction in LVEF (%)	0.21	0.003	0.000–25.572
Increase in Hs cTnT (ng/L)	0.55	0.962	0.829–1.079

GLS, global longitudinal strain; Hs cTnT, high-sensitivity troponin T; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume.

variables (*Figure 5*), values that could early detect patients with cardiotoxicity were selected. One month after completing anthracycline treatment, an LVESV >39.5 mL [sensitivity 57%; specificity 85%; area under the ROC curve (AUC) = 0.75, confidence interval (CI) 0.55–0.96), a GLS value <−20.3 (sensitivity 86%; specificity 71%; AUC = 0.78, CI 0.52–1.0), and an LVEF <60.5% (sensitivity 100%; specificity 66%; AUC = 0.89, CI 0.79–0.99) were chosen as cut-off values to early detect patients with cardiotoxicity.

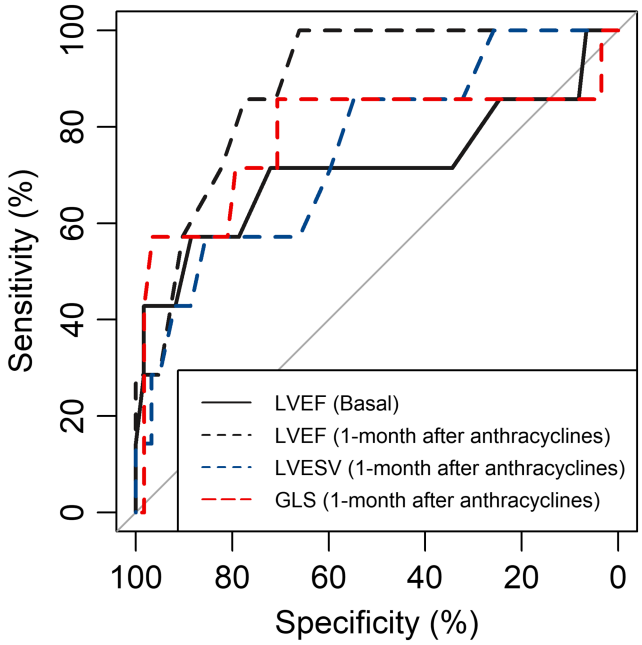
Intra-observer and inter-observer reproducibility of echocardiographic parameters

Measurements recorded twice by each observer obtained an ICC = 0.97 for LVEDV, an ICC = 0.98 for LVESV, and an ICC = 0.93 for LVEF. Measurements recorded by different observers obtained an ICC = 0.92 for LVEDV, an ICC = 0.95 for LVESV, and an ICC = 0.95 for LVEF. Taken together with *Figure S4*, this shows a good reproducibility for echocardiographic parameters.

Discussion

In our prospective cohort of patients with breast cancer treated with anthracyclines ± trastuzumab and with an exhaustive follow-up, we observed that (i) hs cTnT rises gradually over the course of treatment, reaching a maximum peak 1 month after completing anthracycline treatment, but is not able to predict cardiotoxicity; (ii) NT-proBNP does not predict cardiotoxicity development and the values vary greatly

Figure 5 Receiver operative characteristic curve analysis of predictive variables of cardiotoxicity. GLS, global longitudinal strain; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume.



depending on the time of extraction during the anthracycline cycle; (iii) trastuzumab therapy does not affect troponin values but it modifies ventricular volumes; (iv) baseline LVEF is a good predictor of late cardiotoxicity; (v) GLS and LVESV enable early detection of cardiotoxicity, and the best time for their evaluation is 1 month post-anthracycline treatment.

One of the strengths of our study design was the large number of echocardiograms and blood tests performed (6 echocardiograms and 12 blood tests per patient), allowing for an early evaluation of possible alterations in echocardiographic variables and biomarker levels. In fact, this extensive follow-up permitted the detection of cardiotoxicity in the subclinical stage in all patients except one who presented symptoms of heart failure at the time of echocardiographic diagnosis of cardiotoxicity. Early detection and cardioprotective treatment initiation most likely prevented clinical and echocardiographic progression of heart failure in our patients. We propose that the optimum time for early detection of cardiotoxicity by GLS and LVESV is 1 month after completing anthracycline treatment, coinciding with maximum hs cTnT levels. In our study, performing the echocardiography before completing the anthracycline cycles did not contribute any additional relevant data.

The 9.7% cardiotoxicity rate in our series, defined in accordance with the most widely used definition in the consensus document by Plana *et al.*,[8] is similar to recent studies.[14,15] However, this rate is lower than those reported in older studies prior to this document, showing incidences ranging from 18.6% to 32.0%.[10,16,17] These differences are probably linked to a previous broader definition of toxicity, the applied chemotherapy protocol, including epirubicin (88.9% of our cohort), a drug less cardiotoxic than doxorubicin that was used in older series,[18] and the relatively low chemotherapy doses currently recommended as first line therapy for breast cancer.

Another strength of our study is the possibility to correlate imaging and biomarker data given that our study protocol includes hs cTnT. NT-proBNP, LVEF, GLS, and LV volumes. Our results of GLS and LVEF are in line with previous research.[7,10,12,19] However, most of these publications did not include a correlation with hs cTnT. In our series, increased levels of hs cTnT were detected in 62.5% of patients which contrasts with the 9.7% of toxicity detected by echocardiographic parameters; these data reflect a higher sensitivity of the hs cTnT over the imaging techniques used. However, the increase in troponin values is relatively low and the clinical impact of this discrete rise is unknown. Additionally, we describe LVESV as an early detector of cardiotoxicity. This is important in cases where it is not feasible to determine GLS, as myocardial deformation techniques are not always available, they require specially trained staff and a good acoustic window to perform a satisfactory evaluation. In these cases, the evaluation and interpretation of ventricular volumes is essential, and these measurements can be made by all ultrasound systems.

Interestingly, our data contributes to the value of hs cTnT in the cardio-oncology field, which is currently scarce. Serum hs cTnT increased gradually over the course of anthracycline

cycles, reaching a maximum peak at 96 ± 13 days after starting chemotherapy, and showing a similar pattern in patients with or without cardiotoxicity. Moreover, we observed a linear dependence of troponin with age, which was not correlated with the degree of toxicity, so age should also be taken into account when interpreting hs cTnT levels. In other pathologies, such as acute coronary syndrome, the prognostic value of troponins is also affected by age.[20] On the other hand, we found it to be irrelevant whether extractions were carried out before or after each anthracycline cycle, given that levels raised gradually over the course of treatments. Unlike results published in other studies, neither the troponin peak nor the increase in troponin vs. baseline had a predictive value for cardiotoxicity.[10,16] A recent meta-analysis of 61 studies (n = 5691) assessed the value of troponins in cancer patients and found that elevated levels can predict ventricular dysfunction.[9] However, the value of this meta-analysis and its interpretation were limited because only five of the studies measured high-sensitivity troponins. Sawaya *et al*. found that levels of high-sensitivity troponin I >30 pg/mL 3 months after anthracycline treatment predicted cardiotoxicity (sensitivity 48%, specificity 73%). These authors, like ourselves, found no differences in high-sensitivity I troponin between groups with and without cardiotoxicity (32 vs. 17 pg/mL, P = 0.18).[16] On the other hand, Kang *et al*. studied 75 lymphoma patients treated with epirubicin and observed that hs cTnT levels rose after the 3rd treatment cycle (from 0.001 to 0.0063 ng/mL, $P < 0.01$), in agreement with our results, and although the combination of hs cTnT with GLS slightly raised the sensitivity (from 86% to 93%), it was not an independent predictor of cardiotoxicity either.[17] Katsurada *et al*., in a small number of patients (n = 19), observed a rise in high-sensitivity troponin I and T levels 3 months after treatment in both groups, with and without cardiotoxicity; however, significant differences between the groups were only found in hs cTnT levels, and these were only recorded 6 months after the treatment.[21] *Table S2* summarizes the studies that used high-sensitivity troponin.

Regarding NT-proBNP levels, results from post-anthracycline extractions always reached pathological values vs. pre-cycle levels. This demonstrates the importance of the timing of biomarker extraction during chemotherapy treatment and could explain the differences and controversies among previously published studies.[9] The cause of the rise in NT-proBNP at 24–48 hours post-cycle is unknown, although it could be related to volume overload during administration of the drug.[22]

Finally, we observed that trastuzumab-induced toxicity differs from anthracycline-induced cardiotoxicity. As opposed to a gradual rise and 1 month peak of hs cTnT post-anthracycline treatment, trastuzumab does not lead to such rise and dose-dependent pattern. Similar results are described by Zardavas *et al*. in 533 patients in the HERA study (adjuvant herceptin),[23] in which very few patients presented raised troponin levels during treatment with trastuzumab, suggesting that this drug *per se* does not raise troponin levels. They also observed a fall in LVEF in patients who started with raised troponin levels at baseline, but conclude that this rise was secondary to previous anthracycline received. We also found that trastuzumab caused an increase in LVEDV and LVESV, suggesting a different mechanism of ventricular involvement to that produced by the anthracyclines. These data support using ultrasound over biomarkers in patients treated with trastuzumab when evaluating cardiotoxicity.

Limitations

We studied a cohort of young patients with scarce cardiovascular pathology, thus limiting the extrapolation of our results to older patients with a greater cardiovascular risk. However, breast cancer is prevalent in young women, so our findings could be useful to help manage this patient profile. Because our study was conducted in a single centre and a limited number of women developed cardiotoxicity, the findings should be interpreted with caution and confirmed by multicentric studies. Eight out of the 80 patients did not complete the study protocol; but this is in line with previous reported drop-out rates.[16] Moreover, we analysed bidimensional LVEF, with the geometrical limitations this entails. However, the suboptimum acoustic window in mastectomy patients makes difficult the three-dimensional analysis. Finally, the use of additional imaging techniques such as cardiac magnetic resonance, including regional strain or T1 and T2 mapping could add more accurate and sensitive information for the detection of cardiotoxiciy.[24] Nevertheless, these novel techniques are not widely available in many centres, they require higher expertise and analysis might be challenging.

Conclusions

One month after completion of anthracycline treatment is the optimal time to detect cardiotoxicity by means of imaging parameters (LVESV and GSL) and to determine maximal troponin rise. Baseline LVEF was a predictor of later cardiotoxicity whereas hs cTnT and NT-proBNP were not able to predict such chemotherapy side-effect. NT-proBNP values vary greatly depending on the time of extraction during the anthracycline cycle. Trastuzumab therapy does not affect troponin values, but it modifies LVEDV and LVESV; hence, imaging techniques are recommended to detect trastuzumab-induced cardiotoxicity.

Acknowledgements

To Laura Fernández-Friera for her support in patients follow-up.

Funding sources

This work was supported by *Fundación de Investigación HM hospitales* (*I Convocatoria Intramural para grupos emergentes*, 2016). LFF and AM have received funding from *Comunidad de Madrid* (AORTASANA-CM; B2017/BMD-3676), *Fondo Social Europeo (FSE)* and LFF from *Instituto de Salud Carlos III*, España (PI15/02019;PI20/01238).

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Clinical variables, echocardiographic parameters and biomarkers at baseline and over the course of treatment with trastuzumab

Table S2. Studies that evaluate the predictive value of high-sensitivity troponins for chemotherapy-induced cardiotoxicity

Figure S1. Oncology treatment protocols

Figure S2. Echocardiographic parameters at baseline and during follow up of the whole cohort (values expressed as median and interquartile range)

ECHO1: baseline; ECHO2: 2^{nd}-3^{rd} anthracycline cycle; ECHO3: 1^{st} month post-anthracyclines; ECHO4: 3^{rd} month post-anthracyclines; ECO5: 6^{th} month post-anthracyclines; ECO6: 12^{th} month post-anthracyclines; GLS: global longitudinal strain; LVEDV: left ventricular end-diastolic volume; LVEF: left ventricular ejection fraction; LVESV: left ventricular end-systolic volume.

Figure S3. Correlation between High sensitivity troponin T and age

LAB1: 1^{st} anthracycline cycle; LAB2: 2nd anthracycline cycle; LAB3: 3^{rd} anthracycline cycle; LAB4: 4^{th} anthracycline cycle; LAB5: 1^{st} month post-anthracyclines; LAB6: 3^{rd} month post-anthracyclines; LAB7: 6^{th} month post-anthracyclines; LAB8: 12^{th} month post-anthracyclines

Figure S4. Intraobserver and interobserver reproducibility

LVEDV: left ventricular end-diastolic volume; LVEF: left ventricular ejection fraction; LVESV: left ventricular end-systolic volume.

References

1. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005; **365**: 1687–1717.
2. Limat S, Daguindau E, Cahn JY, Nerich V, Brion A, Perrin S, Woronoff-Lemsi MC, Deconinck E. Incidence and risk-factors of CHOP/R-CHOP-related cardiotoxi- city in patients with aggressive non-Hodgkin's lymphoma. *J Clin Pharm Ther* 2014; **39**: 168–174.
3. Herrmann J, Lerman A, Sandhu NP, Villarraga HR, Mulvagh SL, Kohli M. Evaluation and management of patients with heart disease and cancer: cardio-oncology. *Mayo Clin Proc* 2014; **89**: 1287–1306.
4. Zamorano JL, Lancellotti P, Rodriguez Muñoz D, Aboyans V, Asteggiano R, Galderisi M, Habib G, Lenihan DJ, Lip GYH, Lyon AR, Lopez Fernandez T, Mohty D, Piepoli MF, Tamargo J, Torbicki A, Suter TM, ESC Scientific Document Group. 2016 ESC position paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines. *Eur Heart J* 2016; **37**: 2768–2801.
5. Cardinale D, Sandri MT, Martinoni A, Borghini E, Civelli M, Lamantia G, Cinieri S, Martinelli G, Fiorentini C, Cipolla CM. Myocardial injury revealed by plasma troponin I in breast cancer treated with high-dose chemotherapy. *Ann Oncol* 2002; **13**: 710–715.
6. Cardinale D, Sandri MT, Colombo A, Colombo N, Boeri M, Lamantia G, Civelli M, Peccatori F, Martinelli G, Fiorentini C, Cipolla CM. Prognostic value of troponin I in cardiac risk stratification of cancer patients undergoing high-dose chemotherapy. *Circulation* 2004; **109**: 2749–2754.
7. Thavendiranathan P, Poulin F, Lim KD, Plana JC, Woo A, Marwick TH. Use of myocardial strain imaging by echocardiography for the early detection of cardiotoxicity in patients during and after cancer chemotherapy. A systematic review. *J Am Coll Cardiol* 2014; **63**: 2751–2760.
8. Plana JC, Galderisi M, Barac A, Ewer MS, Ky B, Scherrer-Crosbie M, Ganame J, Sebag IA, Agler DA, Badano LP, Banchs J, Cardinale D, Carver J, Cerqueira M, DeCara JM, Edvardsen T, Flamm SD, Force T, Griffin BP, Jerusalem G, Liu JE, Magalhães A, Marwick T, Sanchez LY, Sicari R, Villarraga HR, Lancellotti P. Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: a report from the ASE and the EACVI. *J Am Soc Echocardiogr* 2014; **27**: 911–939.
9. Michel L, Mincu RI, Mahabadi AA, Settelmeier S, al-Rashid F, Rassaf T, Totzeck M. Troponins and brain natriuretic peptides for the prediction of cardiotoxicity in cancer patients: a meta-analysis. *Eur J Heart Fail* 2020; **22**: 350–361.
10. Sawaya H, Sebag IA, Plana JC, Januzzi JL, Ky B, Cohen V, Gosavi S, Carver JR, Wiegers SE, Martin RP, Picard MH, Gerszten RE, Halpern EF, Passeri J, Kuter I, Scherrer-Crosbie M. Early detection and prediction of cardiotoxicity in chemotherapy-treated patients. *Am J Cardiol* 2011; **107**: 1375–1380.
11. Farsalinos KE, Daraban AM, Ünlü S, Thomas JD, Badano LP, Voigt JU. Head-to-head comparison of global longitudinal strain measurements among nine different vendors. The EACVI/ASE inter-vendor comparison study. *J Am Soc Echocardiogr* 2015; **28**: 1171–1181.
12. Negishi K, Negishi T, Hare JL, Haluska BA, Plana JC, Marwick TH. Independent and incremental value of deformation indices for prediction of trastuzumab-induced cardiotoxicity. *J Am Soc Echocardiogr* 2013; **26**: 493–498.
13. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf

FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt JU. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the ASE and the EACVI. *J Am Soc Echocardiogr* 2015; **28**: 1–39.
14. Charbonnel C, Convers-Domart R, Rigaudeau S, Taksin AL, Baron N, Lambert J, Ghez S, Georges JL, Farhat H, Lambert J, Rousselot P, Livarek B. Assessment of global longitudinal strain at low dose anthracycline-based chemotherapy, for the prediction of subsequent cardiotoxicity. *Eur Heart J Cardiovasc Imaging* 2017; **18**: 392–401.
15. Mahjoob MP, Sheikholeslami SA, Dadras M, Mansouri H, Haghi M, Naderian M, Sadeghi L, Tabary M, Khaheshi I. Prognostic value of cardiac biomarkers assessment in combination with myocardial 2D strain echocardiography for early detection of anthracycline-related cardiac toxicity. *Cardiovasc Haematol Disord-Drug Target* 2020; **20**: 74–83.
16. Sawaya H, Sebag IA, Plana JC, Januzzi JL, Ky B, Tan TC, Cohen V, Banchs J, Carver JR, Wiegers SE, Martin RP, Picard MH, Gerszten RE, Halpern EF, Passeri J, Kuter I, Scherrer-Crosbie M. Assessment of echocardiography and biomarkers for the extended prediction of cardiotoxicity in patients treated with anthracyclines, taxanes, and trastuzumab. *Circ Cardiovasc Imaging* 2012; **5**: 596–603.
17. Kang Y, Xu X, Cheng L, Li L, Sun M, Chen H, Pan C, Shu X. Two-dimensional speckle tracking echocardiography combined with high-sensitive cardiac troponin T in early detection and prediction of cardiotoxicity during epirubicine-based chemotherapy. *Eur J Heart Fail* 2014; **16**: 300–308.
18. Mao Z, Shen K, Zhu L, Xu M, Yu F, Xue D, Li H, Xue C. Comparisons of cardiotoxicity and efficacy of anthracycline-based therapies in breast cancer: a network meta-analysis of randomized clinical trials. *Oncol Res Treat* 2019; **42**: 405–413.
19. Narayan HK, Finkelman B, French B, Plappert T, Hyman D, Smith AM, Margulies KB, Ky B. Detailed echocardiographic phenotyping in breast cancer patients associations with ejection fraction decline, recovery, and heart failure symptoms over 3 years of follow-up. *Circulation* 2017; **135**: 1397–1412.
20. Myint PK, Kwok CS, Bachmann M, Stirling S, Shepstone L, Zaman MJ. Prognostic value of troponins in acute coronary syndrome depends upon patient age. *Heart* 2014; **100**: 1583–1590.
21. Katsurada K, Ichida M, Sakuragi M, Takehara M, Hozumi Y, Kario K. High-sensitivity troponin T as a marker to predict cardiotoxicity in breast cancer patients with adjuvant trastuzumab therapy. *Springerplus* 2014; **3**: 620.
22. Ozkan HA, Bal C, Gulbas Z. Assessment and comparison of acute cardiac toxicity during high-dose cyclophosphamide and high-dose etoposide stem cell mobilization regimens with N-terminal pro-B-type natriuretic peptide. *Transfus Apher Sci* 2014; **50**: 46–52.
23. Zardavas D, Suter TM, Van Veldhuisen DJ, Steinseifer J, Noe J, Lauer S, Al-Sakaff N, Piccart-Gebhart MJ, de Azambuja E. Role of troponins I and T and N-terminal prohormone of brain natriuretic peptide in monitoring cardiac safety of patients with early-stage human epidermal growth factor receptor 2-positive breast cancer receiving trastuzumab: a herceptin adjuvant study cardiac marker substudy. *J Clin Oncol* 2017; **35**: 878–884.
24. Giusca S, Korosoglou G, Montenbruck M, Geršak B, Schwarz AK, Esch S, Kelle S, Wülfing P, Dent S, Lenihan D, Steen H. Multiparametric early detection and prediction of cardiotoxicity using myocardial strain, T1 and T2 mapping, and biochemical markers: a longitudinal cardiac resonance imaging study during 2 years of follow-up. *Circ Cardiovasc Imaging* 2021; **14**: 473–484.