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Immediate evaluation of global longitudinal strain

at initiation of trastuzumab treatment in breast cancer patients

Short title: Early strain evaluation in trastuzumab treatment

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Abstract:

Background

Global longitudinal strain (GLS) is recommended to detect subclinical changes preceding reduced left ventricular ejection fraction (LVEF) in trastuzumab related cardiotoxicity. Since the possibility to detect signs of acute myocardial deterioration at treatment initiation is not clarified, the objective of this study was to assess changes in GLS and biomarkers within the first two weeks of trastuzumab treatment.

Methods

In a prospective cohort study 45 patients with non-metastatic breast cancer (age 54, LVEF 62.8%, GLS -19.9%, 40% hypertension) scheduled for trastuzumab treatment were included. Echocardiography and measurement of troponin and NT-proBrain-Natriuretic-Peptide were conducted before initiation of trastuzumab, at day 3, 7 and 14 and after 3, 6 and 9 months.

Results

A significant deterioration in LVEF from 62.8% (SD±3.6) to 58.4% (SD±4.1) ($p<0.0001$), GLS from -19.9 (SD±2.1) to -18.1 (SD±2.5) ($p=0.004$), s' ($p<0.0001$), e' septal ($p=0.008$), and s'RV ($p<0.0001$) occurred at 9 months and was preceded by significant changes in these parameters within the first 14 days.

After 14 days 12 patients (27%) had a ≥10% deterioration in GLS, which was associated with significantly lower LVEF at 55.2% (SD±4.1) at nine months compared to patients with <10% early

deterioration in GLS (LVEF=59.5% (SD±3.5) (p=0.001)). No difference in plasma concentrations of biomarkers was observed between the two groups.

Conclusion

In this study deteriorations in key echocardiographic parameters within normal limits were detected during the first two weeks of trastuzumab treatment, and an early ≥10% deterioration in GLS was associated with a lower LVEF at nine months.

Clinical Trial Registration: ClinicalTrials.gov ID: NCT02440620

Key words: Longitudinal strain, left ventricular function, cardio toxicity, breast cancer, trastuzumab.

Introduction

Between 15 and 30 % of breast cancer tumors express the tyrosine kinase receptor Human Epidermal Growth Factor Receptor 2 (HER2), which is associated with a poor prognosis (1). Trastuzumab is a monoclonal antibody directed against HER2, and survival in non-metastatic HER2 positive breast cancer has been improved with adjuvant trastuzumab therapy (2-4). Trastuzumab has however been associated with cardiac toxicity with development of left ventricular (LV) dysfunction and heart failure. In a meta-analysis of randomized trials testing trastuzumab in an adjuvant setting a 5-fold increased risk of significant reduction of left ventricular ejection fraction (LVEF) with development of symptomatic HF in 1-2 % of the patients was reported (3). However observational studies of clinical unselected populations suggest, that the incidence might be higher up to 20 % (5, 6)

Due to this risk of cardiac toxicity regular evaluation of LVEF is recommended during trastuzumab treatment (7, 8). However, since LVEF is considered a relative crude measure and a significant reduction a late manifestation of cardiac toxicity, early parameters of toxicity has been searched for.

Biomarkers, especially troponins and natriuretic peptides, have been investigated as potentially early markers for myocardial dysfunction during trastuzumab treatment, but their clinical utility has not yet been fully clarified (9, 10). LV deformation analysis using 2-dimensional (2D) speckle tracking has been proposed as a reproducible sensitive measure of LV systolic function (11). A deterioration in global longitudinal strain (GLS) after 3 months of antineoplastic treatment has been shown to precede a reduction in LVEF (12) and is therefore included in the recommendations for cardiac surveillance during potentially cardio-toxic cancer treatment (7, 8, 13).

The possibility of identifying very early signs of myocardial dysfunction at the initiation of trastuzumab treatment has, however, not been thoroughly investigated. Therefore, the aim of this study was to assess immediate manifestations of myocardial injury in echocardiographic parameters and cardiac biomarkers within the first two weeks after initiation of trastuzumab treatment in patients pre-treated with anthracycline based chemotherapy and subsequent trastuzumab.

Methods

This was a single center prospective cohort study of HER2 positive non-metastatic breast cancer patients referred to adjuvant trastuzumab treatment after anthracycline based chemotherapy. Patients were treated between September 2014 and February 2017. Exclusion criteria were inadequate acoustic window on echocardiography, LVEF <50 %, permanent atrial fibrillation or a history of moderate or serve valvular heart disease.

A study examination including a transthoracic echocardiography (TTE), electro cardiogram (ECG), measurement of cardiac troponin T (cTnT) and NT-proBrain Natriuretic Peptide (NT-proBNP) and clinical assessment was conducted immediately before trastuzumab treatment initiation and at day 3, day 7 and day 14 (visit 1-4) after the first dose of trastuzumab. TTE, ECG and clinical assessment

were repeated at follow-up after 3, 6 and 9 months (visit 5-7). The study examinations were blinded for clinicians managing the anti-neoplastic treatment. Data were recorded in a REDCap electronic database hosted at Odense Patient Explorative Network.

The anti-neoplastic treatment consisted of three cycles of epirubicin (90 mg/m^2) and cyclophosphamide (600 mg/m^2) followed by either 3 cycles of docetaxel (100 mg/m^2) every three weeks or 9 weekly cycles of paclitaxel (80 mg/m^2) concomitant with trastuzumab (600 mg). Trastuzumab was administrated subcutaneous and distributed on 17 individual cycles during a year. When indicated by guidelines patients received endocrine and radiation therapy (14). Routine cardiac evaluation comprised a clinical assessment including a standard echocardiogram before chemotherapy treatment and assessment of LVEF by isotope multi gated acquisition (MUGA) scan at weeks 0, 9, 18, 30 and 48 during the trastuzumab treatment. An overview of the study course is presented in figure 1.

Echocardiography

The TTE examinations were performed on an EPIQ 7 ultrasound system (Philips Healthcare) with a 5S transducer by one operator with image optimization to obtain highest possible framerate (>45Hz). Images were stored for blinded analysis on dedicated software for 2D analysis (Xcelera, Philips Healthcare) and myocardial deformation analysis (QLAB 10.3, Philips Healthcare). A second reviewer analysed 10 % of the examinations. In a random sample of 20 echocardiograms (10 in each group) inter- and intra-observer variability of LVEF and GLS was evaluated by repeating these measurements.

From the parasternal long-axis view the diameter of the LV outflow tract (LVOT) was measured as well as LV dimensions (diastolic interventricular septum (IVSd), diastolic LV internal diameter (LVIDd) and posterior wall thickness in diastole (PWd)). LV mass was calculated by the formula 0.8

x (1.04[(IVSd + LVIDd + PWd)3 – (LVIDd)3]) + 0.6 g. Biplane LV end-systolic and end-diastolic volumes and left atrial (LA) maximal volume were measured from apical four- and two-chamber views using the Simpsons method. LA volume measurement was conducted with exclusion of pulmonary veins and LA appendix (15). All volumes were reported indexed for body surface area (BSA). LVEF was evaluated by Simpson's biplane method. By m-mode septal and lateral mitral annular plane systolic excursion (MAPSE) was measured. Stroke volume was calculated by LVOT area x LVOT velocity time integral (VTI).

Deformation analysis was conducted using the speckle tracking software with manual adjustment of myocardial tracking. Maximal longitudinal strain for the 17 LV segments was acquired from 2D cine-loops obtained from the apical two-, four-chamber, and long-axis views. Total GLS was computed and presented in a bulls eye plot.

Assessment of mitral inflow was conducted with pulsed-wave Doppler with the sample volume placed at the tips of the mitral leaflets in diastole. Peak E and A wave velocities and E-wave deceleration time were measured. Early diastolic (e'), atrial (a') and peak systolic (s') mitral annular plane velocity were measured in the septal and lateral mitral annulus using tissue Doppler. Diastolic function was categorized according to guidelines (16). Right ventricular function was evaluated by m-mode with tricuspid annular plane systolic excursion (TAPSE) and peak systolic (s'RV) tricuspid annular plane velocity by tissue Doppler.

Biomarkers

After a minimum of 15 minutes of rest blood was drawn from a peripheral vein at visit 1, 2, 3 and 4. The sample was immediately centrifuged and stored at -80 ^{0}C until analysis at the end of study. cTnT and NT-proBNP were analysed using an electrochemistry-luminescence immunoassay on a Cobas 8000 and a Cobas c602 analyser respectively (Roche Diagnostics). cTnT had a coefficient of variation at internal control at 3.7 % at 21.99 ng/L and detection limits between 3-10000 ng/L. Detection limits for NT-proBNP were 5 and

35 000 ng/L.

End-points

Primary exploratory echocardiographic endpoints were GLS and LVEF estimated by Simpsons biplane method at visit 1 – 7. To investigate if an early increase in GLS was associated with changes in echocardiographic parameters at follow-up visits, patients were divided according to a deterioration of more or less than 10 % 14 days after initiation of trastuzumab relative to GLS measured at the baseline. The 10 % was chosen since a relative 11 % increase in GLS at 6 months was previously reported as a predictor of a more than 10% decline in LVEF at 12 months (17). The secondary endpoint was a permanent or temporary interruption of trastuzumab treatment due to suspected cardiotoxicity.

Statistics

Baseline characteristics were described using proportions for categorical variables and means (± standard deviation (SD)) or medians (interquartile range (IQR)) for continuous variables. Normal distribution was tested graphically by QQ plots and histograms and by the Shapiro-Wilk test. The two sample t-test, the Wilcoxon two-sample test and the Chi-Square test were used for comparison of baseline characteristics and difference between groups as appropriate. In case of numbers less than five Fisher's Exact test was used. A mixed model for repeated measures was used to examine variables during the study period with GLS increase of ≥10 % as fixed effect. A two-sided P-value <0.05 was considered statistical significant.

For statistical analysis and data management the SAS statistical software package, version 9.4. (SAS Institute, Gary, NC) was used.

Ethics

The study was conducted in accordance with the Helsinki Declaration with informed verbal and written consent provided from all participants. Approval by The Committee on Health Research Ethics in the Region of Southern Denmark was obtained (ID: S-20140090) and by the Danish Data Protection Agency of the Region of Southern Denmark (reference: 2008-58-0035). The study was registered ClinicalTrials.gov (ID: NCT02440620).

Results

In total 63 patients were eligible for the study. Five patients were excluded due to inadequate acoustic window (n=4) or significant valve disease (n=1), and further 13 patients did not wish to participate. Thus 45 patients were enrolled in the study.

Mean age was of 53.8 years (SD ± 11.2) with little or no comorbidity beside arterial hypertension, which was seen in 40 % of the patients. A history of cardiac disease was recorded in 3 patients, with one case of ischemic heart disease and two with a single episode of supraventricular arrhythmia. At visit 1 all patients were in sinus rhythm and no arrhythmia or episodes of myocardial ischemia were documented during the study. Baseline clinical characteristics are presented in table 1 for the total cohort and the two groups with GLS deterioration ≥10 % or <10 % from visit 1 to visit 4, respectively.

Prior to the trastuzumab treatment epirubicin treatment was completed in all but one patients (97.8 %). The median dose of epirubicin was 270.0 mg/m^2 (IQR 264.5-275.6). Details on tumor characteristics and antineoplastic treatment are presented in table S1 in supplementary.

Baseline echocardiographic characteristic

LV size and function was normal at visit 1 with a mean LVEF of 62.8 % (SD ± 3.6), which was unchanged since the clinical examination before initiation of chemotherapy (61.8 %, SD ± 2.9). GLS

was -19.9 (SD ± 2.1) at visit 1. Detailed information on baseline echocardiographic parameters is presented in table 2. The inter-observer analysis revealed a mean difference of 3.0 (SD ± 1.7) percent points for LVEF and 0.7 (SD ± 0.7) percent points for GLS. The corresponding percent points for the intra-observer analysis were 1.7 (SD ± 0.9) and 1.0 (SD ± 0.6). Bland-Altman plots of difference from the mean inter- and intra-observer values are presented in Figure S1 and S2 in supplementary.

Time course of changes in echocardiographic parameters and biomarkers

During the study period LVEF decreased significantly from 62.8 % (SD ± 3.6) to 58.4 % (SD ± 4.1) ($p < 0.0001$) and GLS increased from -19.9 (SD ± 2.1) to -18.1 (SD ± 2.5) ($p = 0.004$) for the total cohort. As illustrated in figure 2 the majority of the decrease in LVEF and the increase in GLS occurred within the first 14 days after initiation of trastuzumab. A decrease primarily seen within the first 14 days was also found for e' septal ($p = 0.008$), s' RV ($p < 0.0001$) (figure 2) and mean s' ($p < 0.0001$) (table S2 in supplementary). No significant changes were found in mitral deceleration time (figure 2), E/e' mean (table S2), LV or LA size.

During the first 14 days after initiation of trastuzumab median NT-proBNP decreased from 87.0 ng/L (IQR 39.0 - 116.0) at visit 1 to 44.0 ng/L (24.0 – 109.0) ($p = p<0.0002$) at visit 4 for the total cohort. For cTNT the opposite course was found with an increase from 8.1 ng/L (IQR 5.4-11.3) to 11.4 (IQR 6.6-14.6) ($p = 0.001$). Details on the biomarker measurements are presented in table 3.

Early change in global longitudinal strain

Twelve patients had ≥10 % deterioration in GLS from visit 1 to 4. There was no difference between the two groups with ≥10 % or <10 % deterioration in GLS in baseline demographics, LVEF or in management of breast cancer (table 1, 2 and S1). Patients with ≥10 % increase in GLS deteriorated more in LVEF ($p = 0.001$) during the study period to a value of 55.2 % (SD ± 4.1) at nine months vs.

59.5 % (SD ± 3.5) (p = 0.001) in patients with a GLS increase <10 % (figure 3a and table S2). No difference was found between groups for e' septal (figure 3b), s'RV (figure 3c), mean s' or E/e' mean (table S2). An example of GLS increase during the initial 14 days of the trastuzumab treatment is illustrated in figure 4.

There was no difference in NT-pro-BNP or cTnT plasma concentrations levels between the two groups (table 3). In a secondary analysis biomarkers were divided according to a cut-off level set at the upper quartile of all measurement at visit 4 (107.0 ng/L for NT-proBNP, 14.6 ng/L for cTnT) with no associations with LVEF or GLS observed.

Interruption of trastuzumab treatment

Six patients experienced interruption of the trastuzumab treatment due to suspected cardiac toxicity. Three patients had a significant decrease in LVEF below 50 % at the routine MUGA scan in combination with symptoms of dyspnea, and in one of these patients treatment was interrupted permanently. The remaining three patients experienced asymptomatic LVEF reduction below 50 % on MUGA. All patients except the one with permanent treatment interruption, recovered spontaneously.

Patients with interruption of the treatment had significantly lower LVEF at baseline, while no other differences were found in baseline parameters, GLS, relative change in GLS, cTnT or NT-proBNP within the first 14 days between the patients who experienced interruption and those, who completed without pause (table S3 in supplementary contents). However, the three cases with significant decrease in LVEF below 50 % by MUGA and symptoms of dyspnea during the treatment period all had ≥ 10 % increase in GLS from visit 1 to visit 4, but only one had an elevated NT-proBNP (highest value 1024 ng/l), otherwise biomarker measurements were normal.

Discussion

In this prospective cohort study evaluating early changes in echocardiographic parameters, cTnT and NT-proBNP within 14 days after initiation of adjuvant trastuzumab in breasts cancer patients, three main findings were retrieved. First, a significant deterioration within normal values in LVEF, GLS, s', e' septal and s'RV during the 9 months study period were preceded by significant changes in these parameters within the first 14 days. Secondly, a deterioration of GLS of 10 % or more within the first14 days of trastuzumab treatment was able to identify a significantly lower LVEF within normal range by 9 months. Finally, the study suggested that the very early decrease in GLS was not accompanied by myocardial injury in terms of increased cTnT, even though a minor increase in cTnT, mainly driven by the patient group without GLS deterioration, was statistical significant for the whole population.

Changes in echocardiographic parameters

The changes within normal range in LVEF and GLS found in this study over a nine months treatment period with trastuzumab are in line with other studies (18, 19). An American cohort study from 2017 by Narayan et al. describing details about the echocardiographic changes over a 3-year period among different combinations of anthracycline based chemotherapy and trastuzumab reported a decrease in LVEF of 6.6 % after one year in the group treated with anthracycline and trastuzumb (20). This corresponds well with a decrease of 7.0 % in LVEF after 9 months in the present study.

Studies reporting changes in GLS as a precursor of LVEF decline, typically measure the GLS change at 3 or 6 months after treatment initiation, whereas the current study describes acute changes in biventricular function during the first two weeks of trastuzumab. Acute changes are less well described as only one prior study has investigated changes at the first dose of trastuzumab performing echocardiography at day 1 and 7 in 40 patients and reported an increase in GLS from 19.2% to 17.2 % 7 days after the initial dose of trastuzumab (21).

Our findings suggest a uniform depression of measures of myocardial contraction but with limited direct myocyte injury in terms of cTnT increase. This correlates well with the molecular understanding of trastuzumab toxicity, where the contractile apparatus is depleted for ATP (22). The diastolic parameters are inconclusive in this and other studies (17, 19, 20). Although it would be anticipated that a depression of contractile function would be accompanied by myocardial relaxation abnormalities, this may have been blunted by changes in loading conditions in this population induced by dehydration due to gastrointestinal side effects of chemotherapy (7, 16). In correlation with this no suggestion of increased wall stress could be detected by NT-proBNP in this timeframe.

Echocardiographic changes during trastuzumab treatment cannot be interpreted isolated, since trastuzumab most often is preceded by anthracycline based chemotherapy. Besides being cardiotoxic by themselves (23), anthracyclines possibly also potentiate the myocytes to be vulnerable to trastuzumab, by upregulation of protective HER2 related signal pathways as a response to anthracycline cell damage (24). In this study all but one patient had anthracycline based chemotherapy preceding trastuzumab, which could explain the small, but within normal range, increase in cTnT.

Relative change in GLS

Negishi et al. previously reported an 11 % relative increase in GLS at 6 months as a strong predictor of cardiotoxicity defined as a more than 10% decline in LVEF at 12 months (17). This finding was consisting in a systematic review by Thavendiranathan et al in 2014 (12). The present study extend these findings by suggesting, that a relative increase of ≥10 % in GLS 14 days after the first dose of trastuzumab is associated with a lower LVEF within normal range during the treatment period and with high statistical significance after 6 and 9 month. Thus, it suggests, that very early identification of patients at risk of cardiotoxicity could be possible.

Randomized trials testing primary prevention with ACE-inhibitors and/or beta-blockers in all patients receiving trastuzumab are conflicting (25-27), thus a strategy to select patients for early prevention or a closer follow-up is still warranted. An observational study from Santoro et al. found positive results from a strain guided approach to cardio-protection during breast cancer treatment (28). However, the recent randomized SUCCOUR trial, which tested if GLS-guided selection for cardio protection in high-risk patients could prevent LVEF reduction, did not reach a significant result on the primary endpoint, but found a less profound reduction in LVEF compared to clinical practice (29). Still further clinical implications of this approach and maybe an earlier timing of GLS assessment should be tested in larger randomized populations.

Biomarkers would be another option for this selection, but in a recent study by Ponde et al. neither troponins nor natriuretic peptides were predictive of cardiotoxicity (10), and this present study did not suggest, that biomarkers would aid identification of very early subtle changes in LV function.

Limitations

An important limitation of this study is the restricted size of the cohort and interpretation of the data should be cautious and seen as hypothesis generating. All patients in this study were treated with an anthracycline prior to trastuzumab, which might potentiate the myocytes to the toxicity of trastuzumab. The findings should therefore only be generalized to patients with the same anti-neoplastic regime. Another limitation is the variability of the LVEF measurements, which could be improved by the use of cardiac magnetic resonance imaging (30).

Conclusion

Key echocardiographic parameters regarding myocardial function including GLS, LVEF, e' septal and s'RV deteriorated within the first 14 days after initiation of adjuvant trastuzumab treatment in

breast cancer patients in this prospective cohort study. A 10 % or more deterioration in GLS from baseline to 14 days was able to identify a significantly lower LVEF within normal range by nine months, which could make early GLS change a possible tool to select patients for closer follow-up during treatment. Finally the study suggested that very early deterioration of GLS was not accompanied by myocardial injury.

Acknowledgements

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Data availability

It is not possible within Danish legislation to provide open access to data. Permission to work with study data was obtained by application to The Danish Data Protection Agency with reference: 2008-58-0035. Transmission of data to another party requires an application from that party. Information on this is available at https://www.datatilsynet.dk/english/

Figure legends:

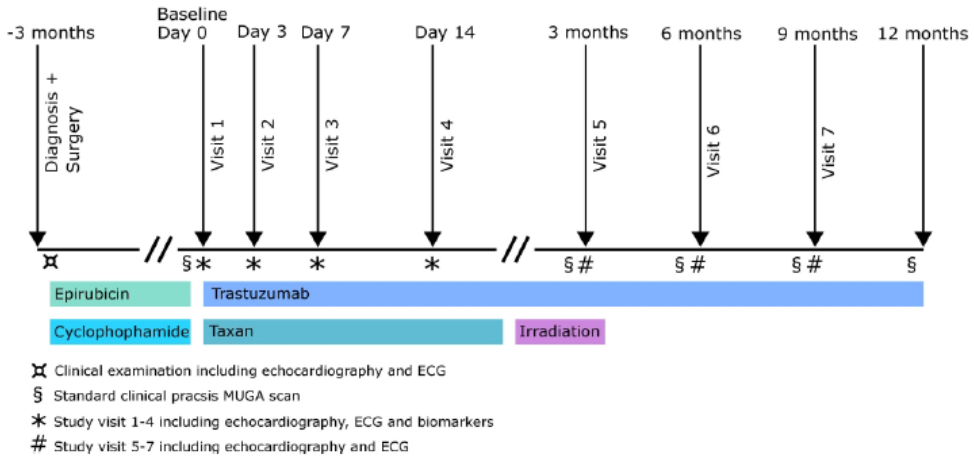

Figure 1:

Overview of the study course including examination modalities at each visit during the treatment period.

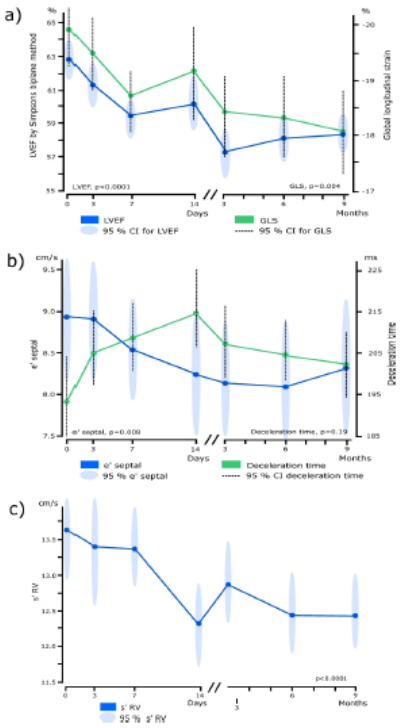

Figure 2:

a) Illustration of an early decrease in LVEF ($p < 0.0001$) and an early increase in GLS ($p = 0.004$) during the study period.

b) Early decrease in e' septal ($p = 0.008$) and deceleration time without significantly change ($p = 0.19$).

c) Early decrease in s'RV ($p<0.0001$).

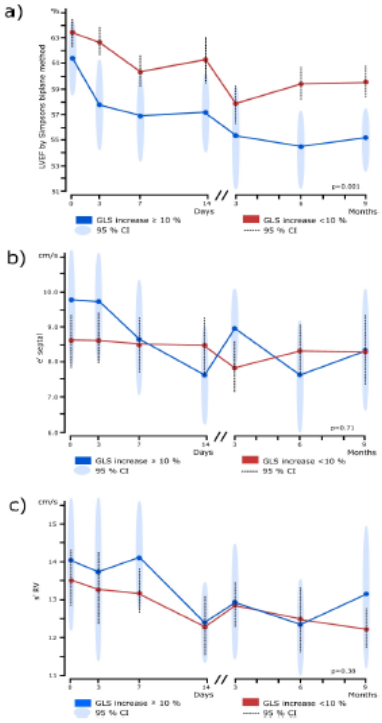

Figure 3:

Difference according to ≥10 % or <10 % increase in GLS from visit 1 to visit 4 in:

a) LVEF (p = 0.001)

b) e' septal (p = 0.71)

c) s'RV (p = 0.38)

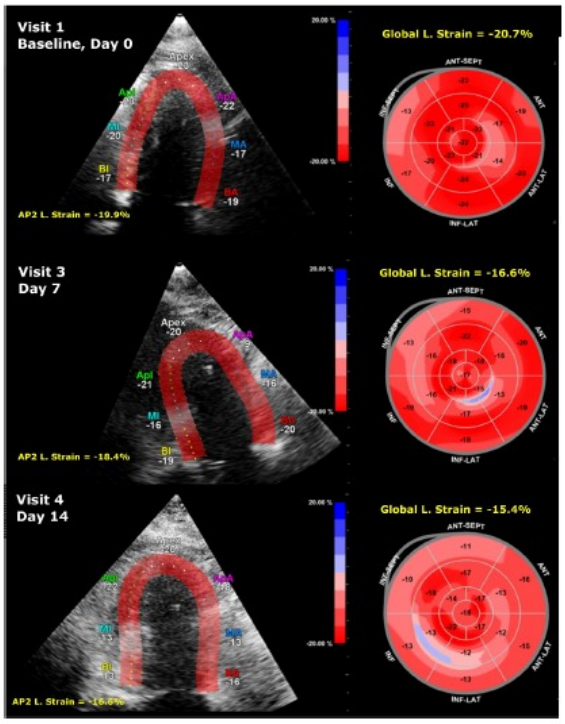

Figure 4:

Illustration of GLS deterioration during the first 14 days of trastuzumab treatment.

Supplementary data

Tables:

Table S1: Presentation of tumor characteristics and oncological treatment for the total cohort and divided according to ≥10 % or <10 % deterioration in GLS from visit 1 to 4.

Table S2: Presentation of selected key echocardiographic variables from all visits for the total cohort and divided according to ≥10 % or <10 % deterioration in GLS from visit 1 to 4.

Table S3: Presentation of mean age, eGFR, BMI, LVEF and GLS from visit 1-4 divided according to interruption or completion without pause in the treatment.

Figures:

Figure S1:

Bland-Altman plots demonstrating difference from inter- and intra-observer means of LVEF.

Figure S2:

Bland-Altman plots demonstrating difference from inter- and intra-observer means of GLS.

Author contributions

All authors have approved of the submitted and final version of the manuscript. Other contributions are listed below:

Ann Banke, MD, Ph.D.: Research design, acquisition, analysis and interpretation of data. Drafting the manuscript.

Morten Schou MD, Ph.D.: Analysis and interpretation of data. Critically revising the manuscript.

Marianne Ewertz, Professor, MD, DMsci.: Research design. Critically revising the manuscript.

Jordi Dahl, MD, DMsci: Research design and interpretation of data. Critically revising the manuscript.

Peter Hartmund Frederiksen, MD: Analysis of data. Critically revising the manuscript.

Lars Videbæk, MD, Ph.D.: Research design and interpretation of data. Critically revising the manuscript.

Søren Cold, MD, Ph.D.: Research design. Critically revising the manuscript.

Jacob E. Møller, Professor, MD, DMSci.: Research design, analysis and interpretation of data. Ststistics. Critically revising the manuscript.

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Tables:

Table 1. Baseline clinical characteristics

Group	All n = 45	GLS increase ≥10 %[a] (n= 12)	GLS increase <10 %[a] (n= 33)	p-value
Mean age	53.8 (SD ± 11.2)	54.1 (SD ± 11.7)	53.7 (SD ± 11.2)	0.91
eGFR[b] mL/min/1.73m2	89 (IQR 77 - 90)	87.5 (IQR 72.0 - 90.5)	89.0 (IQR 81.0 - 90.0)	1.00
Body mass index (BMI) kg/m2	27.3 (IQR 23.3 – 30.1)	28.0 (IQR 22.9 – 31.6)	25.5 (IQR 23.3 – 30.1)	0.83
Smoking: never/previous/current	21/20/4	7/5/0	14/15/4	0.37
Comorbidity:				
Previous heart disease[c]	6.7 % (3)	-	9.1 % (3)	0.55
Hypercholesterolaemia	15.6 % (7)	16.7 % (2)	15.2 % (5)	1.00
Hypertension	40.0 % (18)	50.0 % (6)	36.4 % (12)	0.50
Type 2 diabetes mellitus	4.4 % (2)	8.3 % (1)	3.0 % (1)	0.47
Breast cancer treatment:				
Breast conserving surgery	56.8 % (25)	41.7 % (5)	62.5 % (20)	0.21

Radio therapy	77.8 % (35)	66.7 % (8)	81.8 % (27)	0.42
Endocrine therapy	68.9 % (31)	66.7 % (8)	69.7 % (23)	1.00
Epirubicin, median dose, mg/m2	270.0(IQR 264.5-275.6)	270.0(IQR 264.0-	270.0(IQR 265.0-	0.36

[a]Groups divided according to ≥10 % or <10 % increase in GLS from visit 1 to 4.

[b]Estimated Glomerular Filtration Rate. [c]Arrhythmia n=2 (4.4%), Ischemic n=1 (2.2%).

Table 2. Echocardiographic parameters at baseline

Variables	All n = 45	GLS increase >+10 %[a] (n= 12)	GLS increase <+10 %[a] (n= 33)	p-value
Left ventricle:				
LV[b] mass index g/m^2	80.7 (IQR 71.2-	81.2 (IQR 73.4-89.5)	80.7 (IQR 69.4-85.8)	0.63
LV ED[c] volume index, mL/m^2	48.9 (SD ± 6.1)	50.1 (SD ± 6.7)	48.5 (SD ± 5.8)	0.43
LV ES[d] volume index, mL/m^2	18.1 (SD ± 2.4)	19.2 (SD ± 2.9)	17.7 (SD ± 2.1)	0.07
LVEF[e], Simpson, %	62.8 (SD ± 3.6)	61.4 (SD ± 4.8)	63.3 (SD ± 2.9)	0.11
Global longitudinal strain, %	-19.9 (SD ± 2.1)	-19.9 (SD ± 2.0)	-19.9 (SD ± 2.1)	1.00
MAPSE[f] mean, mm, (n=44)	16.7 (SD ± 1.9)	17.3 (SD ± 2.2)	16.5 (SD ± 1.8)	0.18
s' mean, cm/s	8.6 (SD ± 1.8)	9.7 (SD ± 2.3)	8.2 (SD ± 1.4)	**0.01**
Stroke volume index, ml/m^2,	37.6 (SD ± 4.9)	37.1 (SD ± 5.8)	37.8 (SD ± 4.6)	0.68
LA[g] max volume index, mL/m^2	27.1 (SD ± 5.3)	29.1 (SD ± 6.7)	26.3 (SD ± 4.7)	0.12
Diastolic function				
E velocity, cm/s, (n=44)	76.7 (IQR 68.7-	70.8 (IQR 62.9-95.4)	77.4 (IQR 70.8-91.9)	0.21

A velocity, cm/s, (n=44)	78.8 (SD ± 20.2)	76.5 (SD ± 16.9)	79.7 (SD ± 21.4)	0.64
Deceleration time, ms, (n=43)	193.3 (SD ± 31.5)	178 (SD ± 26)	198 (SD ± 32)	0.07
e' septal, cm/s	8.6 (IQR 6.9-10.4)	8.7 (IQR 7.8-12.0)	8.3 (IQR 6.7-10.0)	0.30
e' mean, cm/s	10.1 (SD ± 2.8)	10.7 (SD ± 2.7)	9.9 (SD ± 2.8)	0.39
a' mean, cm/s	10.0 (IQR 9.2-	10.6 (IQR 9.4-12.8)	9.9 (IQR 8.9-11.4)	0.45
E/e' septal, (n=44)	9.6 (IQR 7.7-10.7)	7.9 (IQR 7.2-9.1)	9.9 (IQR 8.5-11.0)	**0.03**
E/e' mean, (n=44)	8.4 (SD ± 2.4)	7.5 (SD ± 2.2)	8.8 (SD ± 2.4)	0.10
Diastolic dysfunction grade I[h]	6.7 % (3)	8.3 % (1)	6.1 % (2)	1.00
Right ventricle:				
TAPSE[i], mm, (n=44)	28.7 (SD ± 3.7)	29.3 (SD ± 5.0)	28.5 (SD ± 3.2)	0.65
s' RV[j] free wall, cm/s, (n=42)	13.6 (SD ± 2.3)	12.5 (12.4-16.7)	13.2 (12.1-14.8)	0.84

[a]Groups divided according to ≥10 % or <10 % increase in GLS from visit 1 to 4.

[b]LV = left ventricle, [c] ED = end diastolic, [d]ES = end systolic, [e]LVEF = left ventricular ejection fraction, [f]MAPSE= mitral annular plane systolic excursion, [g]LA = left atrium, [h]All other are normal, [i]TAPSE = tricuspid annular plane systolic excursion, [j]RV = right ventricle

Table 3. Biomarkers from all visits for the total cohort and according to GLS increase ≥ or < 10 %

Variables	All n = 45	GLS increase ≥10 %[a] (n= 12)	GLS increase <10 %[a] (n= 33)	p-value[b]
ProBNP, ng/l:				
Baseline	87.0 (IQR 39.0 - 116.0)[c]	62.0 (IQR 33.0-100.0)[d]	89.0 (IQR 44.0-130.0)	0.30
Day 3	62.0 (IQR 38.0 – 126.0)	107.0 (IQR 69.0-259.0)	46.0 (IQR 25.0-119.0)	**0.02**
Day 7	34.5 (IQR 20.0 – 77.0)	30.0 (IQR 10.0-94.0)	37.0 (IQR 21.0-77.0)	0.63
Day 14	44.0 (IQR 24.0 – 109.0)	40.0 (IQR 5.0 -96.0)	58.0 (IQR 30.0-109.0)	0.25
cTNT, ng/l:				
Baseline	8.1 (IQR 5.4-11.3)[e]	7.4 (IQR 4.8-10.7)[f]	8.3 (IQR 5.4-11.8)	0.67
Day 3	8.8(IQR 5.4-13.5)	7.9 (IQR 6.5-8.8)	9.5 (IQR 6.1-14.4)	0.23
Day 7	8.6 (IQR 5.0-12.1)	7.6 (IQR 3.8-12.3)	8.7 (IQR 5.1-12.1)	0.47
Day 14	11.4 (IQR 6.6-14.6)	8.2 (IQR 4.1-12.3)	11.5 (IQR 6.7-16.2)	0.24

[a]Groups divided according to ≥10 % or <10 % increase in GLS from visit 1 to 4.
[b]t test of difference between groups at each visit , [c]p=0.0002 for difference across all visits, [d]p=0.21 for difference between groups across all visits, [e]p=0.001, [f]p=0.12