MONITORING TRASTUZUMAB-INDUCED CARDIOTOXICITY IN HER2-POSITIVE BREAST CANCER

Nathalie I. Bouwer

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Het monitoren van trastuzumab geïnduceerde
cardiotoxiciteit in HER2-positieve borstkanker

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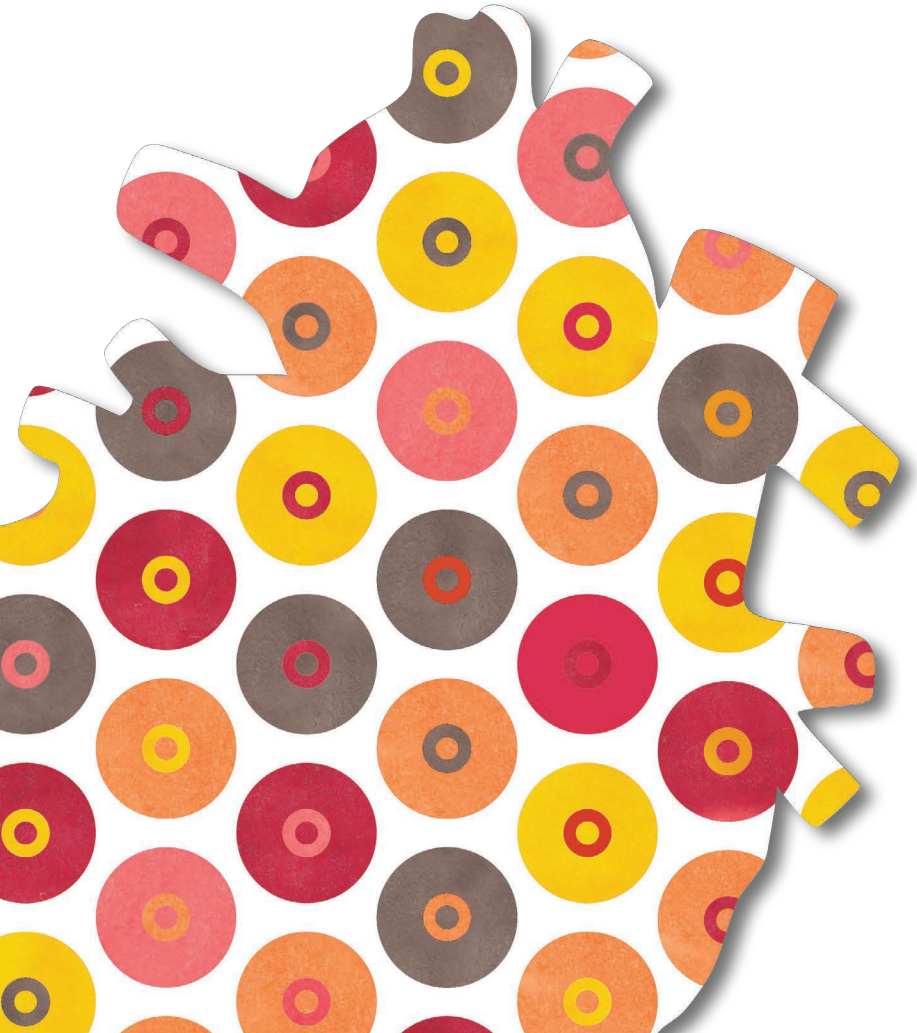

CHAPTER 1

General introduction and outline of thesis

GENERAL INTRODUCTION

Breast cancer

Epidemiology

Breast cancer is the most common cancer in women worldwide.[1] In the Netherlands, in 2019, 15.000 women were diagnosed with invasive breast cancer – the cancer has spread through the lining of the milk ducts into the surrounding tissue – and 2.300 women were diagnosed with non-invasive breast cancer – the cancer has stayed within the ducts or lobules of the breast.[2] The number of breast cancer diagnoses has considerably increased during the past decades, and is still rising (Figure 1). This phenomenon can (partly) be explained by the increased life expectancy, more sensitive detection methods and population screening (above the age of 50 years).[3] As result of early detection and enhanced treatment strategies, the median overall survival of breast cancer has increased from 18 months in 1989 to 34 months in 2015.[2, 4] Nevertheless, female breast cancer is, according to the World Health Organisation (WHO), the fifth leading cause of death worldwide with more than 600.000 deaths (6.6% of all deaths) in 2018.[5] Known risk factors for the development of breast cancer in women include older age, obesity, alcohol use, smoking, early menarche, null parity, older age at first birth, positive family history and genetic predisposition.[6]

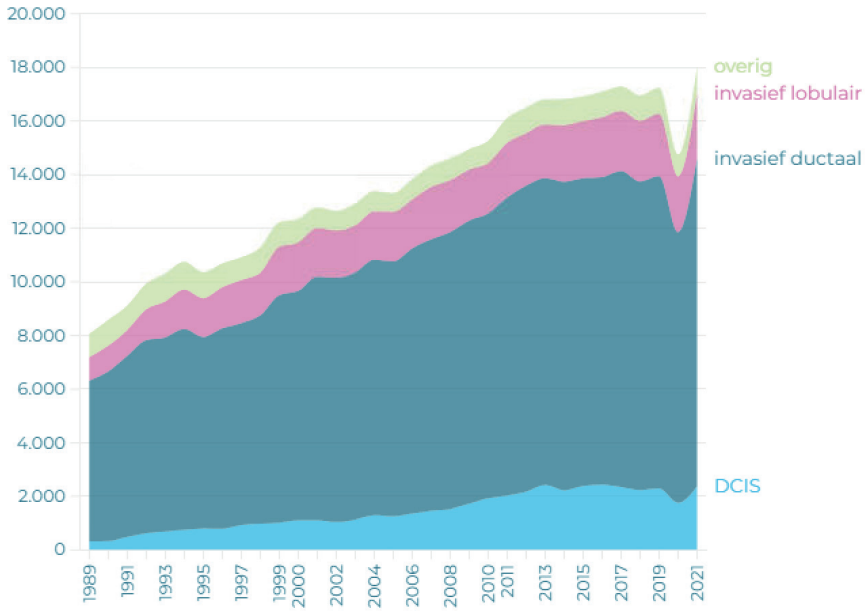

Figure 1. Invasive and non-invasive breast cancer incidence over time*

*As described on *IKNL.nl.*[7]

Treatment

Most patients with breast cancer are diagnosed with early-stage breast cancer (EBC) and in general, they are treated with a curative intent which consists of local therapy (surgery with or without radiotherapy) and perioperative systemic treatment (chemotherapy, and/or endocrine therapy for oestrogen/progesterone receptor positivity, immunotherapy or targeted therapy). Patients with metastatic breast cancer (MBC) cannot be treated curatively and systemic treatment is aimed to prolong survival and maintain or improve the quality of life. Nonetheless, quality of life might be hampered by anti-cancer treatment related toxicities.

Cardiotoxicity

Cardiotoxicity is one of the most important side effects of anti-cancer treatment, including anthracycline and trastuzumab, which results from the unintended effect on cardiomyocytes.[8] This anti-cancer treatment-induced cardiotoxicity often presents as a decline in left ventricular (LV) ejection fraction (LVEF) which potentially can lead to symptomatic heart failure in 1-2% of patients. [9] There is more uncertainty regarding the long-term risk of heart failure, as some trials showed no excess risk of heart failure 2-3 years after trastuzumab initiation. [10-12] However, other observational studies showed a 2-fold increased risk of heart failure with an incidence of up to 20% 5 years after trastuzumab initiation.[13-15] In addition, heart failure caused by anti-cancer treatment has a high mortality of about 60% 2 years after breast cancer diagnosis.[16] Interestingly, trastuzumab-induced cardiotoxicity is believed to be (partly) reversible in 50% while anthracycline is a known cause of irreversible cardiotoxicity.[17, 18] Therefore, early recognition of subclinical cardiotoxicity and prevention of irreversible symptomatic cardiotoxicity is of utmost importance. Indeed, serial cardiac monitoring is recommended, whereas discontinuation of anti-cancer treatment and/or implementation of cardio-protective therapies should also be considered. [18-23] Most importantly, the benefits of continuing anti-cancer treatment should be balanced carefully with the risks of developing cardiotoxicity. The dilemma of continuing anti-cancer treatment to improve the oncological prognosis or discontinuing anti-cancer treatment to maintain the cardiac function plays a central role in this thesis.

HER2-positive breast cancer

The human epidermal growth factor receptor 2 (HER2) is a transmembrane receptor with an intra- and extracellular domain. which plays a role in normal growth and in development of various tissues.[24, 25] Amplification of the HER2-oncogene can lead to overexpression of the HER2, which has shown to play an important role in the rapid growth of HER2-positive breast cancers resulting in a poor prognosis.[26, 27] About 15-20% of all breast tumours have overexpression of the HER2, which has prognostic and predictive implications.[28, 29]

Treatment

HER2-targeted treatment with trastuzumab, which is a humanized monoclonal antibody that binds to the extracellular domain of the HER2, is the cornerstone of treatment for both early-stage and metastatic HER2-positive breast cancer. After binding to the extracellular domain of HER2, trastuzumab inhibits the intracellular tyrosine kinase activity and thereby inhibits the proliferation of HER2-positive breast cancers, resulting in cell death.[30] Current treatment for HER2-positive EBC consists of neoadjuvant or adjuvant treatment with doxorubicin, cyclophosphamide followed by taxane and trastuzumab, and has led to an overall survival of 95% after 7 years.[31] In addition, current treatment for HER2-positive MBC consists of trastuzumab, pertuzumab and chemotherapy and has led to a median overall survival of 56 months.[32] Although HER2-positive MBC is considered an incurable disease, radiological complete remission (rCR) is not uncommon to achieve and some patients remain in complete remission for many years as a result of prolonged trastuzumab treatment.[26, 33-35] However, it remains unknown which characteristics are associated with achieving rCR and improved survival. Furthermore, it is unknown whether trastuzumab can be safely discontinued after achieving rCR to decrease the risk of cardiotoxicity.

Cardiotoxicity of HER2-targeting treatment

The first clinical trials investigating trastuzumab showed an increased risk of cardiotoxicity, which often presented as a LVEF decline.[22] As trastuzumab inhibits the HER2, which is physiologically expressed on cardiomyocytes too, trastuzumab can lead to cardiotoxicity (Figure 2). Studies show that trastuzumab inhibits the cardiomyocyte repair by blocking neuregulin-1 and the HER2 downstream pathway which is required for cardiac repair,[8, 36] and which is especially important after anthracycline treatment.[37]

Most studies use the definition of cardiotoxicity used by the European Society of Cardiology (ESC) of cardiotoxicity related to cancer therapeutics defined as an LVEF decline of >10%-points to a LVEF <50%.[38] However, the United Kingdom National Cancer Research Institute used LVEF <45% and/or an absolute LVEF decline of >10%-points relative to baseline as threshold to interrupt trastuzumab treatment and start heart failure medication.[39] The European Society of Medical Oncology (ESMO) uses a LVEF threshold of 40% to initiate heart failure medication.[40] Apparently, consensus on the definition of cardiotoxicity (and the need for cardio-protective action) is missing. In this thesis, we will take this into account by investigating these various definitions.

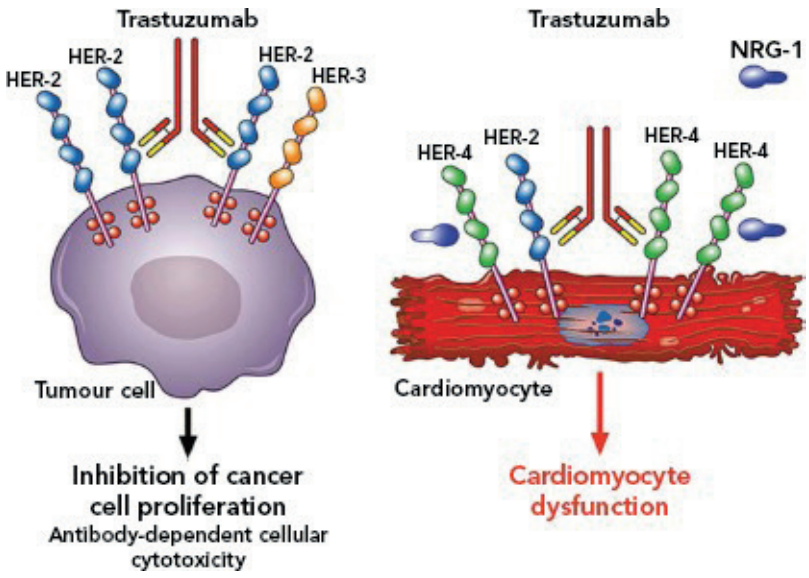

Figure 2. Mechanism of action of trastuzumab on the HER2-positive breast cancer cell and cardiomyocyte**

**Adopted from *Cuomo et al.*[41]

Epidemiology

The reported incidence of cardiotoxicity can be as high as 27% when trastuzumab is combined with doxorubicin and cyclophosphamide.[42] This is the reason why doxorubicin and trastuzumab are currently given sequentially. Interestingly, concomitant use of trastuzumab and epirubicin induces less often cardiotoxicity, with an incidence of only 4%.[43] In addition, it has been shown that longer duration of trastuzumab, i.e. 2 years compared to 1 year for HER2-positive EBC, increases the incidence of cardiotoxicity after 11 years of follow-up (7.3% compared to 4.4%).[44] The incidence of cardiotoxicity during long-term treatment for HER2-positive MBC has only been scarcely investigated.

Risk factors for cardiotoxicity in relation to trastuzumab treatment are older age (>65 years), hypertension, diabetes mellitus, obesity (BMI >30 kg/m^2), previous anthracycline exposure, short time between radiation therapy and compromised cardiac function before treatment.[40, 45-48] Interestingly, a recent study in patients with a compromised cardiac function at baseline (LVEF <50%) showed that with optimized cardio-protective medication, including beta blockers and ACE inhibitors, only 3 patients (10%) developed cardiotoxicity defined as symptomatic heart failure or an LVEF decline >10%-points from baseline and/or LVEF ≤35%.[49] However, safe

initiation of long-term trastuzumab for MBC could not be assessed as the maximum duration of trastuzumab administration was 12 months, and only 13 (42%) patients had HER2-positive MBC.

Cardiac imaging modalities

A strategy of cardiac monitoring during trastuzumab treatment in combination with early discontinuation of trastuzumab and/or early implementation of cardio-protective therapies may positively impact cardiac outcome.[18-23] Monitoring of the cardiac function during trastuzumab is important for early detection of cardiotoxicity. Current cardiac monitoring consists of LVEF measurements with multigated acquisition (MUGA) scan and/or two-dimensional echocardiography (2DE). However, both modalities have some important limitations.

Another sensitive and promising imaging modality is two-dimensional speckle tracking echocardiography (2DE-ST) that can measure strain.[50] Dedicated speckle tracking (ST) software is capable to track the movement of speckles, which are tracking points that are placed automatically on the LV in the 2DE images, and thereby measuring strain (Figure 3). Strain is a method for measuring regional myocardial deformation, i.e. the proportional change of the heart.[51] Positive global radial strain (GRS) represents thickening of the LV wall (radial deformation) and negative strain represents LV wall segment shortening, which can occur as longitudinal shortening (global longitudinal strain) or circumferential shortening (global circumferential strain). Additionally, ST can also be applied in three-dimensional echocardiography (3DE) and in cardiac MRI (CMR). However, knowledge about the associations of these techniques with 2DE-ST is missing. Therefore, ST with 3DE and CMR are currently not frequently performed in clinical practice. It has been showed that a decline in global longitudinal strain (GLS) measured with 2DE-ST precedes an LVEF decline measured with 2DE in patients with HER2-positive breast cancer treated with anthracycline and trastuzumab.[52-55] However, it remains unknown whether addition of ST to conventional 2DE can improve the performance of 2DE to predict LVEF changes measured with CMR.

Optimal frequency and duration

The current recommendation from the US Food and Drug Administration (FDA) label of trastuzumab is that cardiac monitoring should occur every 3 months during and upon completion of adjuvant trastuzumab treatment.[57] After completion of 1 year adjuvant trastuzumab, cardiac monitoring is recommended every 6 months for at least 2 years. If trastuzumab is withheld for significant left ventricular cardiac dysfunction, cardiac monitoring should occur monthly. These recommendations have been adopted into

clinical practical guidelines of the ESMO [40], ESC [38] and American Society of Clinical Oncology (ASCO).[45] More research is needed to investigate the optimal frequency and duration of cardiac monitoring during trastuzumab treatment for HER2-positive EBC and MBC.

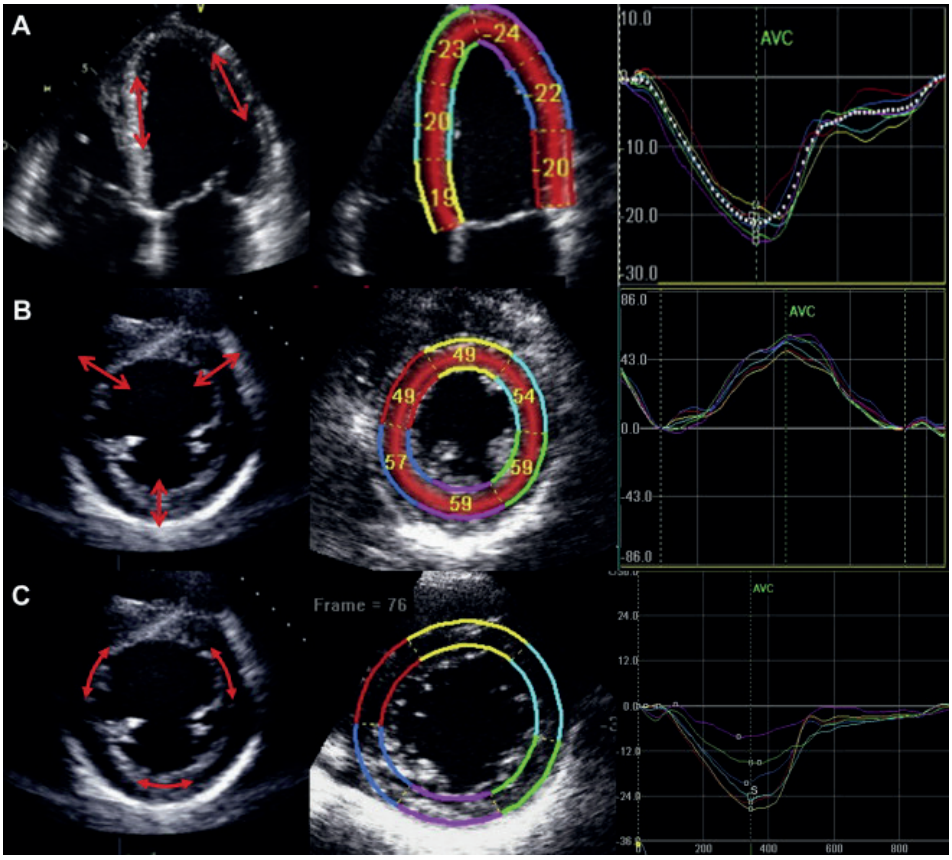

Figure 3. Speckle tracking echocardiography-based strain measurements in a patients with breast cancer. A. global longitudinal strain, B. global radial strain and C. global circumferential strain***

***Adopted from *Thavendiranathan et al.* [56]

Biomarker monitoring strategies

Measuring cardiac biomarkers including troponin, N-terminal fragment of the pro-hormone brain natriuretic peptide (NT-proBNP), troponin C-reactive protein (CRP), myeloperoxidase (MPO), immunoglobulin E (IgE) and suppressor of tumorgenicity 2 (ST2), may also be a potential diagnostic tool for identification of cardiotoxicity as it reduces the use of invasive diagnostic tools, radiation exposure, costs, is less-time consuming for patients and

may possibly detect myocardial damage at an earlier stage than conventional imaging strategies. First, NT-proBNP, which is a well-known biomarker for the identification of heart failure [58], can also be useful for identification of cardiotoxicity during trastuzumab treatment. Studies investigating the association between NT-proBNP and cardiotoxicity in breast cancer patients showed inconclusive results; 2 prospective studies observed increased NT-proBNP levels in patients with cardiotoxicity [59, 60], but in several other studies this was not observed.[55, 61-64].The potency of a screening strategy using repeatedly measured NT-proBNP to detect cardiotoxicity has not been yet investigated. Second, troponin regulates the contractile element actin and myosin in the cardiomyocyte and is elevated in case of myocardial damage due to acute coronary syndrome or myocarditis.[65, 66] It is plausible that high-sensitive (hs) troponin assays may detect trastuzumab-induced cardiotoxicity. Third, CRP is a marker of inflammation and seems to be elevated in chronic heart failure.[67] Inflammation could also play a role in trastuzumab-induced cardiotoxicity. Fourth, MPO is a pro-inflammatory enzyme that is secreted by polymorph nuclear neutrophils and it is involved in the release of oxidative stress.[68, 69] As the pathophysiological mechanism of cardiotoxicity due to trastuzumab might involve oxidative stress formation [70], it is biologically plausible that elevated MPO levels can be associated with the development of trastuzumab-induced cardiotoxicity. Fifth, IgE are antibodies produced by the immune system and are involved in allergic reactions and defence against parasitic diseases.[71] It is known that the immune system is involved in maintaining myocardial homeostasis in patients with heart failure and therefore it could be potential elevated in patients with trastuzumab-induced cardiotoxicity.[72] Last, ST2 reflects cardiovascular stress and myocardial fibrosis.[73] Therefore, it is a relative new prognostic biomarker in heart failure and could also be useful for the identification of trastuzumab-induced cardiotoxicity. However, it is not yet known what the role is of each individual cardiac biomarker for the identification of cardiotoxicity in patients with HER2-positive breast cancer treated with trastuzumab in clinical practice.

Reverse cardio-oncology: cancer development in patients with arterial thrombosis

As mentioned before, anti-cancer treatment can lead to cardiac diseases. However, cardiac diseases, for instance arterial thrombotic events, may also be related to the development of cancer the other way around.[74, 75] This reverse cardio-oncology phenomenon is observed in patients with arterial thrombotic events (for instance ST-segment-elevation myocardial infarction (STEMI)) who have, based on population-based studies, an increased risk of untreated malignancies.[76-80] This risk may even be larger in the first few months after the event which may be explained by shared risk factors (i.e. smoking, obesity, diabetes mellitus and/or alcoholism [81-83]), consequences of the STEMI diagnosis and/or treatment (asymmetrical follow-up) or the possibility of STEMI as a paraneoplastic phenomenon of undiagnosed cancer. However, the possibility of STEMI as a paraneoplastic phenomenon of underlying cancer has been scarcely investigated.

OUTLINE OF THIS THESIS

Against the previously described background and remaining challenges within the field of Cardio-Oncology, the aims of this thesis were three-fold: to evaluate the epidemiology of trastuzumab-induced cardiotoxicity during HER2-positive MBC (1), to optimize cardiac monitoring during trastuzumab treatment (2), and to study cancer development in patients with arterial thrombosis for a better understanding of reverse cardio-oncology (3).

1. Incidence of trastuzumab-induced cardiotoxicity in patients with HER2-positive MBC
In the first part, the yearly incidence of and risk factors for trastuzumab-induced cardiotoxicity during long-term trastuzumab treatment for HER2-positive MBC are studied. By combining the incidence of cardiotoxicity over time with an individual cardiovascular risk profile, a tailored cardiac monitoring recommendation could be given. We investigated cardiotoxicity defined as LVEF decline >10%-points from baseline to LVEF <50%, and LVEF <40%, as a consensus definition of cardiotoxicity is missing. It is likely that the risk of cardiotoxicity is higher in patients with HER2-positive MBC compared to patients with HER2-positive EBC due to longer duration of trastuzumab treatment, the higher prior cumulative doses of anthracycline, other concomitant treatments and relatively older patient population with multiple comorbidities and possibly a compromised baseline LVEF.[19, 22, 44] Therefore, we also investigated whether trastuzumab could be safely started in patients with HER2-positive MBC with a compromised baseline LVEF, i.e. baseline LVEF <50%. Additionally, the role of cardio-protective medication during trastuzumab treatment was described in this vulnerable population. Importantly, the decision to continue trastuzumab treatment despite cardiotoxicity should be weighed against the potential benefits of trastuzumab treatment in patients with HER2-positive MBC. The poor survival of patients with HER2-positive MBC (22% after 5 years) compared to patients with HER2-positive EBC (ranging from 77% to 97% after 5 years) may influence this benefit/risk ratio and the willingness to continue trastuzumab despite development of cardiotoxicity.[84] However, in case of achieving rCR during trastuzumab treatment , the overall survival of patients with HER2-positive MBC is impressively improved.[85] Therefore, we studied clinical characteristics associated with achieving rCR and described the effect of trastuzumab discontinuation after achieving rCR.

2. Cardiac monitoring during trastuzumab treatment of patients with HER2-positive BC
In the second part, an overview and critical appraisal of the state-of-the-art of cardiac monitoring strategies in trastuzumab-treated HER2-positive breast cancer patients is presented, that covers the role, frequency, duration and type of cardiac monitoring along with its current limitations. Additionally, the relationship between strain imaging

with 2DE-ST and CMR has been studied in order to investigate whether addition of ST to conventional 2DE can improve the performance of 2DE for LVEF prediction. This is important as the correlation between CMR, the gold standard for cardiac function evaluation, and 2DE is only poor to moderate and the use of CMR for cardiac monitoring of patients with breast cancer is hampered by its limited availability and high costs.[86, 87] Furthermore, the temporal pattern of NT-proBNP in patients with HER2-positive breast cancer is described during 1-year of trastuzumab treatment. This has been performed by repeatedly non-invasively measured blood samples, which gives an insight into the value of NT-proBNP for cardiotoxicity risk prediction. NT-proBNP was selected as a suitable biomarker, as it is an important biomarker for the identification of heart failure and potentially also of trastuzumab-induced cardiotoxicity.[58]

3. Cancer development in patients with arterial thrombosis

In the third part, the risk of a cancer diagnosis after an acute STEMI over time was described using a hospital-based cohort and large population-based cohort with validated information about potential confounders in order to assess whether a STEMI is associated to underlying cancer as a paraneoplastic phenomenon. In addition, indicators of short-term cancer incidence among STEMI patients were investigated. This is relevant to increase physicians' awareness of a STEMI as a possible paraneoplastic phenomenon of underlying undiagnosed cancer.

Finally, in the fourth part, the main findings of this thesis are discussed, as well as future perspectives and challenges for the optimal care of patients with HER2-positive breast cancer during and after cardio-toxic trastuzumab treatment.

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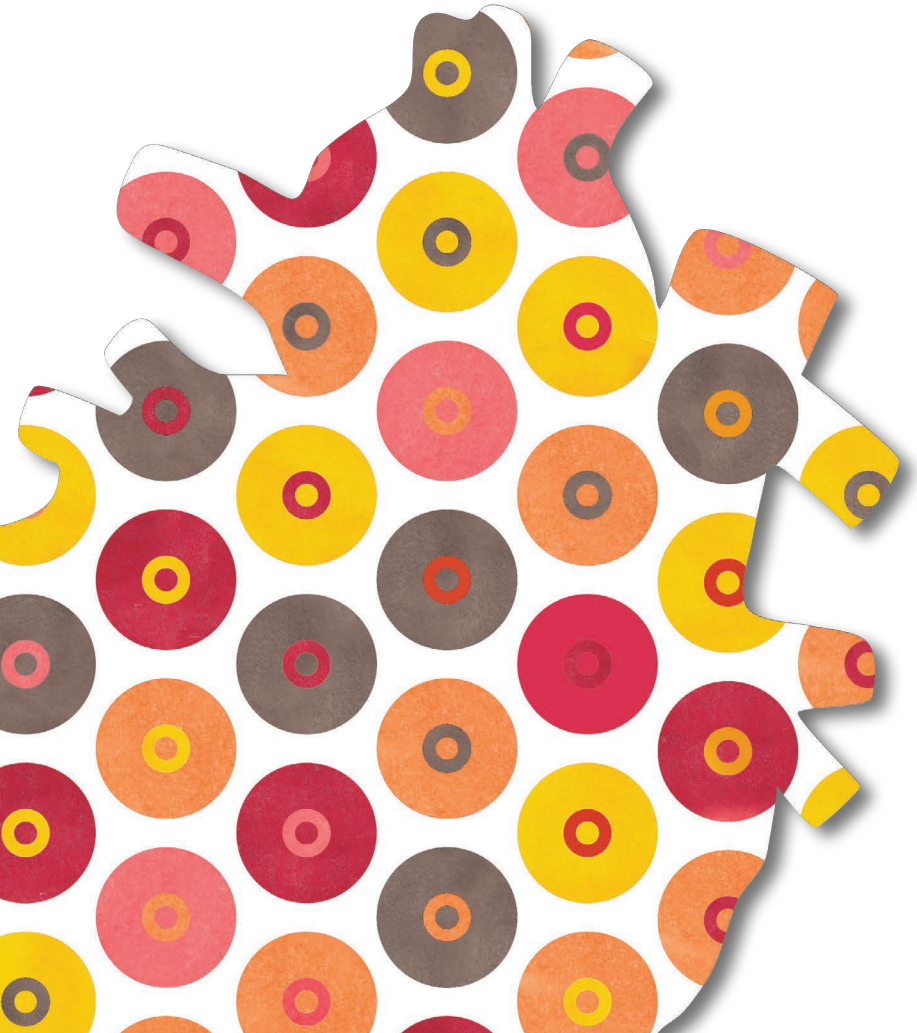
PART I

CARDIOTOXICITY OF TRASTUZUMAB FOR HER2-POSITIVE METASTATIC BREAST CANCER



CHAPTER 2

Cardiotoxicity during long-term trastuzumab use in patients with HER2-positive metastatic breast cancer: who needs cardiac monitoring?

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ABSTRACT

Background
Patients with HER2-positive metastatic breast cancer (MBC) usually receive many years of trastuzumab treatment. It is unknown whether these patients require continuous left ventricular ejection fraction (LVEF) monitoring. We studied a real-world cohort to identify risk factors for cardiotoxicity to select patients in whom LVEF monitoring could be omitted.

Methods
We included patients with HER2-positive MBC who received >1 cycle of trastuzumab-based therapy in eight Dutch hospitals between 2000 and 2014. Cardiotoxicity was defined as LVEF<50% that declined >10%-points and was categorized into non-severe cardiotoxicity (LVEF 40-50%) and severe cardiotoxicity (LVEF<40%). Multivariable Cox and mixed model analyses were performed to identify risk factors associated with cardiotoxicity. Additionally, we explored the reversibility of cardiotoxicity in patients who continued trastuzumab.

Results
In total, 429 patients were included. Median follow-up for cardiotoxicity was 15 months (interquartile range 8–31 months). The yearly incidence of non-severe + severe cardiotoxicity in the first and second year were 11.7% and 9.1%, respectively, which decreased thereafter. The yearly incidence of severe cardiotoxicity was low (2.8%) and stable over time. In non-smoking patients with baseline LVEF ≥60% and no cardiotoxicity during prior neoadjuvant/adjuvant treatment, the cumulative incidence of severe cardiotoxicity was 3.1% after four years trastuzumab. Despite continuing trastuzumab, LVEF decline was reversible in 56% of patients with non-severe cardiotoxicity and in 33% with severe cardiotoxicity.

Conclusions
Serial cardiac monitoring can be safely omitted in non-smoking patients with baseline LVEF ≥60% and without cardiotoxicity during prior neoadjuvant/adjuvant treatment.

INTRODUCTION

Trastuzumab is a monoclonal antibody targeting the human epidermal growth factor receptor 2 (HER2) that has greatly improved the outcome of patients with HER2-positive breast cancer in both the primary and metastatic setting.[1-3] Trastuzumab toxicity is generally mild, although left ventricle ejection fraction (LVEF) decline (cardiotoxicity) is a well-known side effect that is mostly seen in combination with concurrent or sequential anthracycline treatment.[3] Regular LVEF monitoring at a three-monthly interval is therefore recommended during one year of neoadjuvant and/or adjuvant trastuzumab treatment, however during metastatic treatment no specific time interval of LVEF monitoring is recommended.[4] Since the median overall survival of patients with HER2-positive metastatic breast cancer (MBC) is well over four years with continuous use of trastuzumab, the cumulative burden of LVEF monitoring can be high.[5, 6] The incidence of cardiotoxicity during long-term treatment for HER2-positive MBC, however, is not well-known and neither are risk factors for trastuzumab-associated cardiotoxicity in this setting.

Two studies investigated cardiotoxicity over time during trastuzumab treatment in patients with MBC.[7, 8] The first study found a cumulative incidence of cardiotoxicity of 12.7% and 28.5% after 1 and 3 years of trastuzumab use, respectively.[7] They defined cardiotoxicity as LVEF decline >20%-points from baseline or LVEF <50% or symptoms of congestive heart failure. The LVEF recovered in a vast majority of the patients (84%) after discontinuation of trastuzumab with or without cardio-protective treatment. However, reversibility of cardiotoxicity after continuation of trastuzumab has not been described yet. A second study observed a cumulative incidence of cardiotoxicity of 5.3% after 3 years of trastuzumab use. However, they used a composite endpoint that included myocardial ischemia, heart failure, rhythm disorder, and other cardiac diseases.[8] Data on long-term sequelae were not available. Lastly, risk factors for developing cardiotoxicity during long-term trastuzumab treatment could be similar to those causing cardiotoxicity during 1 year of trastuzumab treatment [9-11], however this has not been investigated yet.

Therefore, we studied cardiotoxicity during long-term trastuzumab treatment in patients with HER2-positive MBC in an observational historic multicenter cohort study, and risk factors associated with cardiotoxicity in this setting to select patients in whom LVEF monitoring could be omitted. Additionally, we evaluated the reversibility of cardiotoxicity in patients who continued trastuzumab.

METHODS

Patients and data collection

We included patients with HER2-positive MBC who received >1 cycle of trastuzumab-based treatment in one of eight participating Dutch hospitals between January 2000 and December 2014, as described before.[12] Patients were identified using the Netherlands Cancer Registry. Patients were excluded in case no baseline LVEF measurement was available within 30 days before the first trastuzumab administration for MBC, baseline LVEF <50%, no follow-up LVEF measurements were available during trastuzumab use, and in case of incomplete clinical data in the medical records.

Trained investigators systematically retrieved data on patient and tumour characteristics, treatment, and LVEF measurements from medical records. Medical Ethics Commission of all participating hospitals approved this comprehensive data collection.

Endpoints

We defined non-severe + severe cardiotoxicity and severe cardiotoxicity based on guidelines of the European Society of Cardiology (ESC)[13] and the European Society of Medical Oncology (ESMO)[4], respectively. First, *non-severe + severe cardiotoxicity*, is defined as 1) LVEF decline >10%-points from baseline and LVEF <50% measured with multigated acquisition (MUGA) scan or 2) decline from good/normal cardiac function to at least mild cardiac dysfunction and at least mild cardiac dysfunction measured with echocardiography if MUGA was not available. After a decline in LVEF found with MUGA scan, in some cases this was followed by an echocardiography to exclude false negative low LVEF measurements. In case both investigations were performed, echocardiography was used to define cardiotoxicity. Second, *severe cardiotoxicity*, is defined as 1) LVEF <40% measured with MUGA scan or 2) moderate or severe cardiac dysfunction measured with echocardiography if MUGA was not available.[13] Since patients with LVEF <50% at baseline were excluded, patients with LVEF <40% had by definition a LVEF decline of >10%-points compared to baseline. Lastly, *non-severe cardiotoxicity* was defined as 1) LVEF <50% but ≥40% or mild cardiac dysfunction measured with echocardiography. The time to non-severe + severe cardiotoxicity, non-severe cardiotoxicity or severe cardiotoxicity was calculated from start of trastuzumab treatment for MBC to the first occurrence of non-severe + severe cardiotoxicity.

For the analyses of reversibility, non-severe + severe cardiotoxicity was categorized into non-severe cardiotoxicity and severe cardiotoxicity. Reversibility of cardiotoxicity was defined as any LVEF increase to a value <5% below baseline, partially reversibility

as any absolute LVEF increase ≥10%-points from nadir and to a value >5%-points below baseline, and irreversibility as any absolute LVEF increase <10%-points from nadir and to a value >5%-points below baseline.[14] The frequency of LVEF measurements was determined by the treating physician.

Statistical analyses

Continuous variables are presented as medians with interquartile range (IQR) for non-normal distribution, and as means with standard deviations (SD) for normal distribution. Categorical variables are presented as percentages.

Median follow-up for cardiotoxicity was calculated from start of trastuzumab for MBC until 6 months after last trastuzumab dose or until last LVEF measurement, whichever came first. Discontinuation of trastuzumab treatment was defined as any stop or interruption of trastuzumab treatment. The yearly incidence of cardiotoxicity was investigated. Patients were at risk for cardiotoxicity when receiving LVEF measurements during trastuzumab treatment. After the first development of non-severe cardiotoxicity, patients were no longer at risk for non-severe cardiotoxicity. However, these patients were still at risk for severe cardiotoxicity. After developing severe cardiotoxicity, patients were no longer at risk for any type of cardiotoxicity.

We used univariable and multivariable Cox proportional hazards analyses to determine which baseline variables were associated with non-severe + severe cardiotoxicity and severe cardiotoxicity, and to find a group of patients at low risk of cardiotoxicity. All variables were determined at start of trastuzumab treatment for MBC. Independent variables statistically significant at 0.10 level in univariable analysis or known risk factors for cardiotoxicity from literature were included in the multivariable analysis. The Cox proportional hazards assumption was verified using the Schoenfeld residuals test and was not violated. Multivariable cause-specific Cox proportional hazards models were built with similar variables to investigate potential competing risk from death with non-severe + severe cardiotoxicity or severe cardiotoxicity. In this analysis, patients are censored in case of death due to any cause.

The following variables were included in the analyses: age, BMI, hypertension, diabetes mellitus, smoking, history of cardiac disease, baseline LVEF <60%, cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline, prior cumulative anthracycline exposure, radiation exposure to the breast. BMI was categorized in BMI <25 kg/m^2, 25-30 kg/m^2, and >30 kg/m^2. Hypertension was defined as a history of systolic blood pressure >130 mmHg or diastolic blood pressure >80 mmHg or the use of antihypertensive medication.[15] History of cardiac disease was defined as the

history of either arrhythmia, cardiac valve deficiency, cardiomyopathy or coronary artery disease. Cardiotoxicity during prior treatment was defined LVEF decline >10%-points to a LVEF <50% during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline. Prior cumulative anthracycline exposure was defined as the number of courses anthracycline before trastuzumab in palliative setting. Radiation exposure was categorized in left-sided, right-sided or unknown side. Last, de novo MBC was defined as metastatic disease at time of diagnosis or development of metastases within 3 months of diagnosis.

For the multivariable Cox proportional hazards analyses, missing information on diabetes mellitus, hypertension, smoking, history of cardiac disease, radiotherapy side and cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline was imputed using fully conditional specification with 100 imputations. Estimates were pooled over imputed data sets using Rubin's rules. A sensitivity analysis with a complete case analysis was conducted to investigate robustness of the imputation procedure. In the cause-specific Cox proportional hazards analyses, missing values were imputed using substantive model compatible fully conditional specification with 100 imputations. Additionally, sensitivity analyses were performed by investigating the number of LVEF measurements as a risk factor for cardiotoxicity in a Cox proportional hazards analysis to investigate detection bias. LVEF measurements up until the development of cardiotoxicity were taken into account in these analyses.

To study the relation between the independent variables and continuous LVEF measurements during total follow-up, linear mixed effects model analysis was conducted. Risk factors that are related to a LVEF decline, but not with the definition of cardiotoxicity, were investigated in this analysis. In addition to the variables used in the Cox proportional hazards analysis, this model allows to investigate time varying variables, namely the cumulative trastuzumab exposure at each LVEF measurement calculated from start trastuzumab to each LVEF measurement (in months). The fit of the linear mixed effects models was adjusted by the non-linear curve observed from the predicted values plot (data not shown).[16] The effect of time since start trastuzumab was modelled using restricted cubic splines, with the number of knots (4) chosen using information criteria. Random intercept and random slope of time since start of trastuzumab were included to account for within-patient correlations between repeated measurements.

Inverse Kaplan-Meier curves stratified for significant risk factors were used to investigate the cumulative incidence of non-severe + severe cardiotoxicity and severe cardiotoxicity for the number of significant risk factors from the Cox proportional hazards analysis.

Data analyses were performed using SPSS (version 24.0) and R (version 3.4.3), in particular the packages "lme" , "splines", "JointAI", "smcfcs" and "mice".

RESULTS

Patient characteristics

Between January, 2000 and December, 2014, 745 patients with HER2-positive MBC were identified in the 8 participating Dutch hospitals. After excluding patients who had no baseline LVEF measurement (n=193), a baseline LVEF measurement <50% (n=41), no LVEF measurement during trastuzumab treatment (n=55), or in whom no additional data collection was possible (n=18), 429 patients were eligible for current analyses (Supplementary Figure S1). In general, no differences were observed between included and excluded patients, except for a lower percentage of patients receiving prior neoadjuvant/adjuvant trastuzumab in the latter (Supplementary Table S1). Patient and treatment characteristics are shown in Table 1. Of all patients, 311 (72%) had metachronous distant metastases and 118 (28%) had synchronous distant metastases at disease presentation.

Patients were followed for cardiotoxicity with a median of 15 months (IQR 8–31). The median overall survival for all patients was 42 months (IQR 25–71). Median frequency of LVEF monitoring was 4 times annually (IQR 3–5) with a median total number of LVEF measurements of 4 times during follow-up (IQR 2-7). Most used cardiac imaging modality for LVEF assessment was the MUGA scan in 358 patients (83%). Echocardiography (4%) and CMR (0.2%) or a combination of both (13%) were performed less often.

Incidence of non-severe + severe and severe cardiotoxicity

During total follow-up, 94 patients (22%) developed non-severe + severe cardiotoxicity. In the first year of trastuzumab treatment the incidence of non-severe + severe cardiotoxicity was 11.7% (Figure 1). The yearly incidence gradually decreased over the following years (i.e. 9.1% in year 2 to 3.6% in year 6). The median time to develop non-severe + severe cardiotoxicity from start of trastuzumab for MBC was 11 months (IQR 5–23).

In total, 25 patients (6%) developed severe cardiotoxicity. In the first year of trastuzumab treatment the incidence of severe cardiotoxicity was 2.8% (Figure 1). The yearly incidence of severe cardiotoxicity the next years remained stable (i.e. 1.9% in year 2 to 2.4% in year 6). The median time to severe cardiotoxicity was 10 months (IQR 6–25).

Table 1. Baseline characteristics of all included patients (n=429)

Clinical and treatment characteristics	**No. (%), median [IQR]**
Age (years)[a]	54 [46–61]
Hormonal receptor status[b]	
Positive	235 (55)
Negative	172 (40)
Unknown	22 (5)
Neoadjuvant/adjuvant chemotherapy	
No	213 (50)
Anthracycline + trastuzumab	65 (15)
Anthracycline without trastuzumab	120 (28)
Trastuzumab without anthracycline	19 (4)
Other	12 (3)
Duration of neoadjuvant/adjuvant trastuzumab (months)	12 [12–12]
Prior cumulative anthracycline exposure (courses)[a, c, d]	0 [0–4]
Adjuvant radiotherapy	
No	171 (40)
Left side	126 (29)
Right side	100 (23)
Side unknown	32 (8)
Cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline	
No	329 (77)
Yes	15 (4)
Unknown	85 (20)
Baseline LVEF (%)[a]	
≥60	198 (46)
<60	231 (54)
BMI (kg/m^2)[a]	
<25	164 (49)
25-30	130 (30)
>30	44 (10)
Unknown	91 (21)
History of cardiac disease	
No	379 (88)
Yes	35 (8)
Unknown	15 (4)
Diabetes mellitus[a]	
No	387 (90)
Yes	28 (7)
Unknown	14 (3)

Table 1. Continued

Clinical and treatment characteristics	No. (%), median [IQR]
Hypertension[a]	
No	314 (73)
Yes	98 (23)
Unknown	17 (4)
Hypercholesterolemia[a]	
No	210 (49)
Yes	37 (9)
Unknown	182 (42)
Smoking[a]	
No	203 (47)
Yes	90 (21)
Unknown	136 (32)

Abbreviations: IQR, interquartile range; LVEF, left ventricle ejection fraction; BMI, body mass index; MUGA scan, multigated acquisition scan; MRI, magnetic resonance imaging; MBC, metastatic breast cancer.

[a] At start of palliative trastuzumab treatment; for definition see methods section.

[b] Estrogen and progesterone receptor positivity was defined as ≥ 10% positive nuclear staining [28].

[c] Number of courses before palliative trastuzumab treatment.

[d] Could consist of doxorubicin or epirubicin courses.

Risk factors associated with non-severe + severe cardiotoxicity and severe cardiotoxicity

Risk factors independently associated with non-severe + severe cardiotoxicity were BMI >30 kg/m^2 (adjusted [a]HR 2.16, 95% CI 1.15–4.06), smoking (aHR 1.73, 95% CI 1.05–2.85), cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline (aHR 4.48, 95% CI 1.56–12.87). Prior neoadjuvant/adjuvant trastuzumab (aHR 0.38, 95% CI 0.18–0.82) was associated with less non-severe + severe cardiotoxicity (Table 2). Risk factors independently associated with severe cardiotoxicity were smoking (aHR 6.15, 95% CI 2.12–17.82), baseline LVEF <60% (aHR 7.64, 95%CI 1.70–34.43) and cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline (aHR 5.60, 95% CI 1.03– 30.42, Table 3).

Cause-specific Cox proportional hazards analyses, taking competing risk between cardiotoxicity and death into account, showed similar risk factors associated with non-severe + severe cardiotoxicity and severe cardiotoxicity (Supplementary Table S2).

Table 2. Risk factors present at start of trastuzumab treatment for MBC associated with developing non-severe + severe cardiotoxicity

Age (years)

BMI (kg/m^2)
  <25
  25-30
  >30

Hypertension
Diabetes mellitus
Smoking
History of cardiac disease
Baseline LVEF(%)   ≥60   <60
Prior neoadjuvant/adjuvant trastuzumab
Cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline
Cumulative anthracycline exposure (total number of courses)[b]

Adjuvant radiotherapy
  No
  Left side
  Right side
  Side unknown

De novo metastatic breast cancer

Abbreviations: PH, proportional hazards; HR, hazard ratio; CI, confidence interval; BMI, body mass index; LVEF, left ventricle ejection fraction; MBC, metastatic breast cancer; REF, reference category.

	Univariable Cox PH[a]			Multivariable Cox PH[a]	
HR	95% CI	P-value	Adjusted HR	95% CI	P-value
1.01	0.99 – 1.02	0.574	1.01	0.99 – 1.03	0.254
REF					
1.29	0.76 – 2.17	0.340	1.24	0.73 – 2.12	0.423
2.05	1.13 – 3.73	0.018	2.16	1.15 – 4.06	0.017
1.23	0.77 – 1.95	0.386			
1.20	0.55 – 2.64	0.651			
1.89	1.13 – 3.16	0.016	1.73	1.05 – 2.85	0.031
1.36	0.71 – 2.60	0.352			
REF					
1.60	1.05 – 2.44	0.030	1.52	0.97 – 2.40	0.071
0.60	0.33 – 1.10	0.097	0.38	0.18 – 0.82	0.014
2.36	1.05 – 5.30	0.037	4.48	1.56 – 12.87	0.005
1.05	0.98 – 1.14	0.155	1.05	0.97 – 1.15	0.247
REF					
1.01	0.62 – 1.65	0.977			
1.00	0.57 – 1.73	0.987			
1.22	0.57 – 2.62	0.615			
0.78	0.48 – 1.26	0.313	0.81	0.47 – 1.40	0.451

[a] Based on multiple imputations with MICE of diabetes mellitus, hypertension, smoking, history of cardiac disease, local radiotherapy of the breast and prior cardiotoxicity during treatment with trastuzumab or anthracycline, where death is a censoring event.

[b] A course consist of doxorubicin 60 mg/m^2 or epirubicin 100 mg/m^2.

Table 3 Risk factors present at start of trastuzumab treatment for MBC associated with developing severe cardiotoxicity

Age (years)

BMI (kg/m^2)
  <25
  25-30
  >30

Hypertension
Diabetes mellitus
Smoking
History of cardiac disease
Baseline LVEF (%)   ≥60   <60
Prior neoadjuvant/adjuvant trastuzumab
Cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline
Cumulative anthracycline exposure (total number of courses)[b]

Adjuvant radiotherapy
  No
  Left side
  Right side
  Unknown side

De novo metastatic breast cancer

Abbreviations: PH, proportional hazards; HR, hazard ratio; CI, confidence interval; BMI, body mass index; LVEF, left ventricle ejection fraction; MBC, metastatic breast cancer; REF, reference category.

	Univariable Cox PH[a]			Multivariable Cox PH[a]	
HR	95% CI	P-value	Adjusted HR	95% CI	P-value
1.00	0.97 – 1.04	0.926			
REF					
1.02	0.76 – 2.17	0.975			
1.99	1.13 – 3.73	0.188			
1.39	0.57 – 3.35	0.468			
0.65	0.09 – 4.82	0.673			
6.61	2.23 – 19.60	<0.001	6.15	2.12 – 17.82	<0.001
2.61	0.98 – 6.96	0.056			
REF					
9.91	2.34 – 42.05	0.002	7.64	1.70 – 34.43	0.008
1.07	0.40 – 2.87	0.891			
3.30	0.87 – 12.56	0.079	5.60	1.03 – 30.42	0.045
1.21	1.07 – 1.37	0.003	1.15	1.00 – 1.33	0.051
REF					
1.01	0.41 – 2.53	0.975			
0.73	0.23 – 2.31	0.597			
0.56	0.07 – 4.34	0.577			
0.46	0.16 – 1.34	0.153			

[a] Based on multiple imputations with MICE diabetes mellitus, hypertension, smoking, history of cardiac disease, local radiotherapy of the breast and prior cardiotoxicity during treatment with trastuzumab or anthracycline, where death is a censoring event.

[b] A course consist of doxorubicin 60 mg/m^2 or epirubicin 100 mg/m^2.

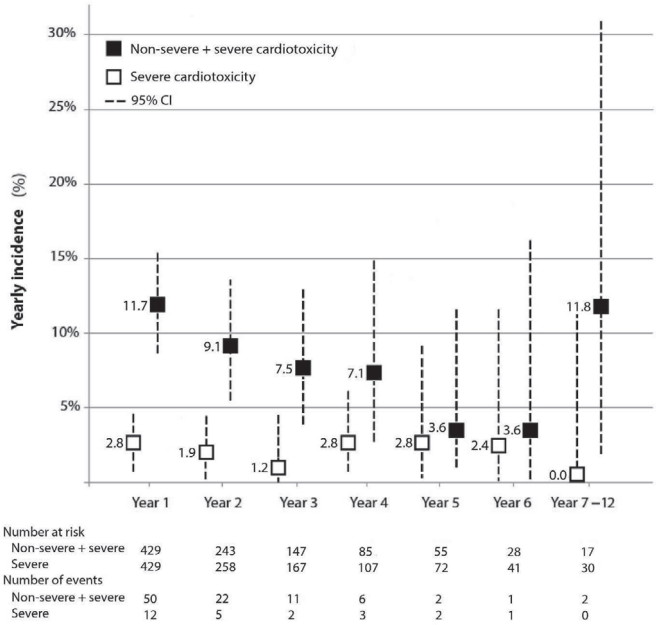

Figure 1. Yearly incidence of non-severe + severe cardiotoxicity and severe cardiotoxicity during trastuzumab treatment

Abbreviations: CI, confidence interval.

Linear mixed effects model analysis (Table 4) also showed similar risk factors that were associated with LVEF differences over time as the Cox proportional hazards analyses (Tables 2 and 3). This means that patients who smoked on average had 2.77%-points lower LVEFs at the same time point compared to patients who did not smoke (Table 4; $p<0.001$).

Cumulative incidence of cardiotoxicity per relevant risk factors

The identified significant risk factors from the Cox proportional hazards analyses (Tables 2 and 3) were used to identify a patient group at low risk for cardiotoxicity, regardless of their effect size. In total, 241 patients had no relevant risk factors, 152 patients 1 risk factor and 36 patients 2 or 3 risk factors. Regarding relevant risk factors for severe cardiotoxicity, 242 had no risk factors, 158 patients 1 risk factors and 29 patients 2 or 3 risk factors. Patients without relevant risk factors for severe cardiotoxicity had a low cumulative incidence of 3.1% after a total follow-up of 4 years (Figure 2). The cumulative incidence for both non-severe + severe cardiotoxicity and severe cardiotoxicity increases in case of more relevant risk factors.

Sensitivity analyses evaluating detection bias of cardiotoxicity

Patients who received 0-2 LVEF measurements annually had lower risk of non-severe + severe cardiotoxicity and severe cardiotoxicity compared to patients who received 3-4 LVEF measurements annually (HR 0.43, 95% CI 0.24–0.78), however not of severe cardiotoxicity (HR 0.66, 95% CI 0.22–1.97). Additionally, patients who received >4 LVEF measurements annually had higher risk of non-severe + severe cardiotoxicity (HR 6.39, 95% CI 3.57–11.41) and severe cardiotoxicity (HR 3.65, 95% CI 1.38–9.62).

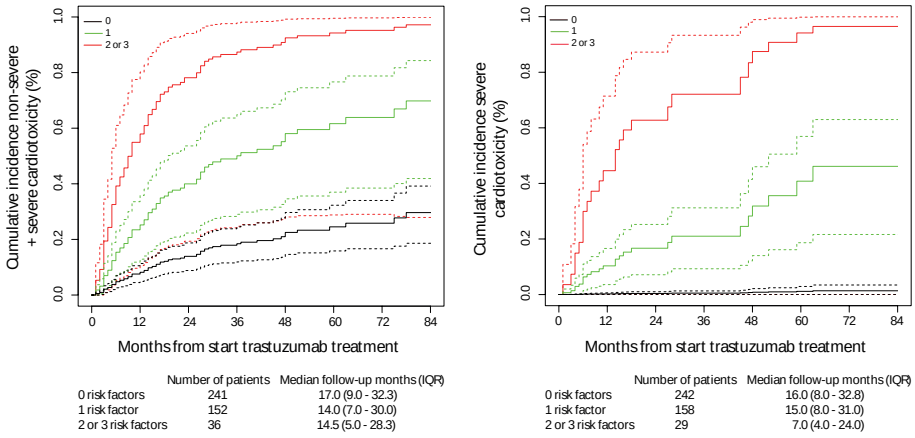

Figure 2. Cumulative incidence of non-severe + severe and severe cardiotoxicity with respect to the number of relevant risk factors

Solid lines indicate cumulative incidence; dashed lines indicate corresponding 95% CI of the cumulative incidence. For non-severe + severe cardiotoxicity, the following risk factors were included: BMI >30 kg/m^2, smoking and cardiotoxicity during prior neoadjuvant/ adjuvant treatment with trastuzumab and/or anthracycline (Table 2). For severe cardiotoxicity the following risk factors were included: smoking, cardiotoxicity during prior neoadjuvant/ adjuvant treatment with trastuzumab and/or anthracycline and baseline LVEF <60% (Table 3).

Reversibility of non-severe + severe cardiotoxicity and severe cardiotoxicity

To explore the reversibility of cardiotoxicity, we categorized the patients who developed non-severe + severe cardiotoxicity into non-severe cardiotoxicity (n=69) and severe cardiotoxicity (n=25), of whom reversibility could be analysed in 58 patients with non-severe cardiotoxicity and 16 patients with severe cardiotoxicity (Figure 3).

Table 4. Risk factors present at start of trastuzumab for MBC associated with continuous LVEF differences at each time point during total follow-up

Cumulative trastuzumab exposure (months)[b]
Age (years)

BMI (kg/m²)
  <25
  25 – 30
  >30

Hypertension
Diabetes mellitus
Smoking
History of cardiac disease
Baseline LVEF(%)   ≥60   <60
Prior neoadjuvant/adjuvant trastuzumab
Cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline
Cumulative anthracycline exposure (total number of courses)[c]

Adjuvant radiotherapy
  No
  Left side
  Right side
  Side unknown

De novo metastatic breast cancer

Abbreviations: LVEF, left ventricle ejection fraction; CI, credible interval; BMI, body mass index; MBC metastatic breast cancer; REF, reference category.

Estimated absolute LVEF difference at each time point (%)[a]	95% CI	Tail-probability
0.27	-0.06 to 0.51	0.163
0.02	-0.05 to 0.09	0.642
REF		
-1.04	-2.90 to 0.73	0.237
-1.09	-3.44 to 1.30	0.389
0.67	-1.13 to 2.47	0.461
-0.71	-3.62 to 2.14	0.624
-2.77	-5.26 to -0.66	0.013
0.40	-2.21 to 3.04	0.766
REF		
-6.72	-8.17 to -5.28	<0.001
2.11	-0.09 to 4.31	0.060
-13.70	-22.64 to -5.45	<0.001
-0.38	-0.69 to 0.08	0.012
REF		
0.20	-1.61 to 2.02	0.827
0.44	-1.54 to 2.43	0.662
1.46	-1.38 to 4.26	0.307
1.39	-0.45 to 3.24	0.144

[a] Based on multiple imputations with MICE of diabetes mellitus, hypertension, smoking, history of cardiac disease, local radiotherapy of the breast and prior cardiotoxicity during treatment with trastuzumab or anthracycline.

[b] From start of palliative trastuzumab treatment to each LVEF measurement.

[c] A course consist of doxorubicin 60 mg/m^2 or epirubicin 100 mg/m^2.

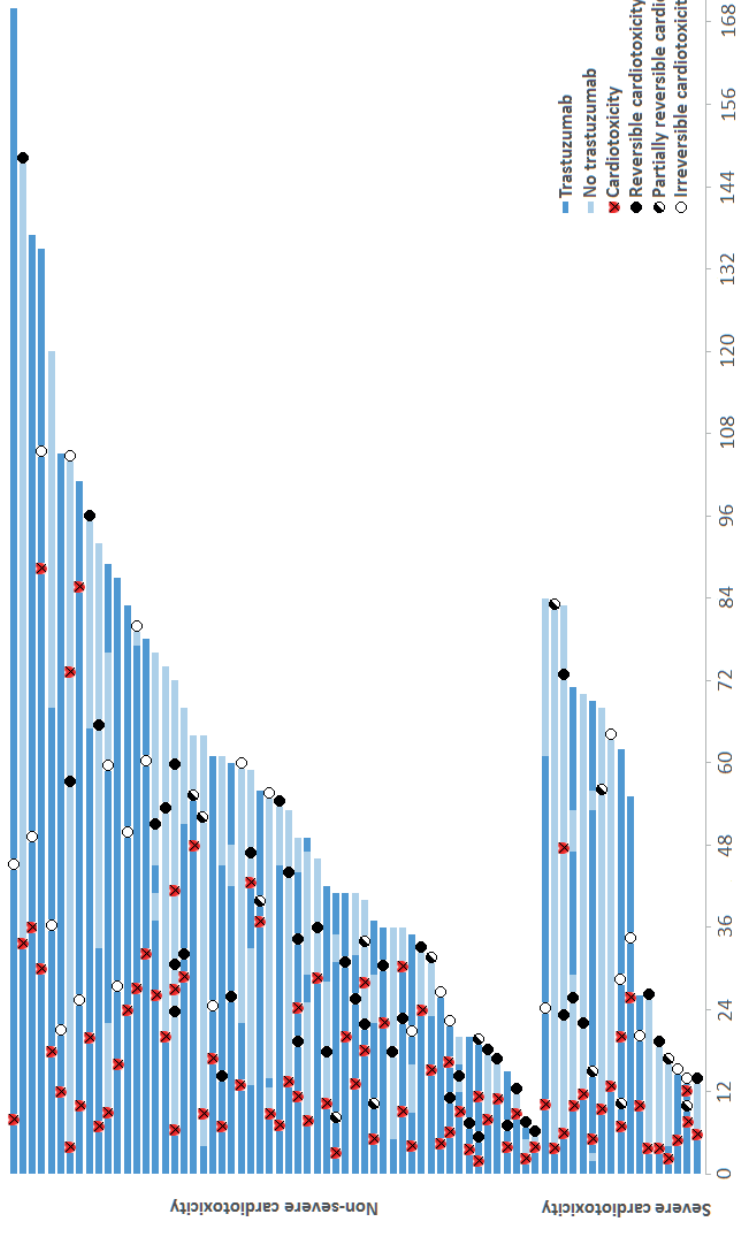

Figure 3. Swimmersplot of all patients who developed cardiotoxicity and received LVEF measurements thereafter (n=74)

*Whichever occurred last. Reversibility of non-severe and severe cardiotoxicity was defined as any LVEF increase to a value <5% below baseline value, partially reversibility as any LVEF increase ≥10 from nadir and to a value >5% below baseline value and irreversibility as an absolute LVEF increase <10% from nadir and to a value >5% below baseline value.[14]

In total, the LVEF decline was reversible in 34 out of 58 patients (59%) who developed non-severe cardiotoxicity (Figure 3 and Supplementary Figure S2). Among the 15 patients who discontinued trastuzumab for at least one cycle after non-severe cardiotoxicity, 7 patients received LVEF measurements. Of those patients, the LVEF was reversible in 4 patients (57%), partially reversible in 1 patient (14%) and irreversible in 2 patients (29%). Of the 43 patients who continued trastuzumab, 23 patients (56%) had normalisation of LVEF, in 6 patients (15%) the LVEF decline was partially reversible and in 12 patients (29%) irreversible. Of all patients developing non-severe cardiotoxicity, 23% had cardiac symptoms including shortness of breath or angina pectoris. The reversibility was independent of the presence of cardiac symptoms.

The LVEF decline was reversible in 6 out of the 16 patients (35%) who developed severe cardiotoxicity and received LVEF measurements (Figure 3 and Supplementary Figure S2). Among the 19 patients who discontinued trastuzumab after severe cardiotoxicity, the LVEF decline was reversible in 4 patients (40%), partially reversible in 3 patients (30%) and irreversible in 3 patients (30%). Among the 6 patients who continued trastuzumab after severe cardiotoxicity, the LVEF decline was reversible in 2 patients (33%), partially reversible in 3 patients (50%) and irreversible in 1 patient (17%). In the five patients with reversible severe cardiotoxicity, trastuzumab could be continued safely without recurring cardiotoxicity for a mean duration of 17 months (range 3–35). Of all patients who developed severe cardiotoxicity, 72% had cardiac symptoms. The reversibility was independent of the presence of cardiac symptoms.

DISCUSSION

In this study, we showed that among patients with HER2-positive MBC the yearly incidence of non-severe + severe cardiotoxicity was highest in the first 2 years of trastuzumab (11.7% and 9.1%, respectively) and gradually decreased over time. The median time to develop non severe + severe cardiotoxicity was 11 months. The yearly incidence of severe cardiotoxicity was low over time (range 1.2%-2.8%) with a median time to develop severe cardiotoxicity of 10 months. In non-smoking patients with baseline LVEF ≥60% and no cardiotoxicity during prior neoadjuvant/adjuvant treatment (i.e. no relevant risk factors), the cumulative incidence of severe cardiotoxicity was limited to 3.1% after 4 years trastuzumab. Physicians often continued trastuzumab treatment despite cardiotoxicity, i.e. 62% in case of non-severe cardiotoxicity and 24% in case of severe cardiotoxicity. Interestingly, reversibility was relatively high among those who continued trastuzumab (71% after non-severe cardiotoxicity and 83% after

severe cardiotoxicity of whom 56% and 33% fully recovered, respectively). Taken together, our data shows the limited clinical relevance of regular cardiac monitoring by LVEF measurements in patients without relevant risk factors, stressing the need for an alternative monitor schedule.

The incidence of cardiotoxicity observed in this study was lower than reported in some other studies investigating cardiotoxicity of trastuzumab in the palliative setting.[3, 7, 18] This might be explained by the fact that these studies included patients treated with concomitant and higher doses of anthracycline than our study.[3, 18] In addition, some studies used other imaging modalities besides MUGA scan and had a less strict criteria to define cardiotoxicity.[7, 18]

We aimed to identify subgroups with particular low risk of developing cardiotoxicity in order to tailor cardiac monitoring. In our study, high BMI, smoking, cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline and baseline LVEF <60% were found to be statistically significant independent risk factors for cardiotoxicity, whereas other cardiovascular risk factors were not. This might be due to the fact that our study population consisted of relatively young patients (median age was 54 years) with a good LVEF (>50%) before starting trastuzumab. In the study of Rossi *et al.*, age was an important risk factor for cardiotoxicity among patients receiving trastuzumab for HER2-positive MBC.[8] In the study of Guarneri *et al.*, baseline LVEF and time from last anthracycline administration were important risk factors.[7] In addition, a recent study indicated that polymorphism HER2-Ile655 A>G is a risk factor for developing cardiotoxicity during trastuzumab.[19] Whether this risk factor will help personalizing cardiac monitoring in patients with HER2-positive MBC remains to be investigated.

By combining the incidence of cardiotoxicity over time with an individual cardiovascular risk profile, a tailored cardiac monitoring recommendation could be given, in case a yearly incidence of severe cardiotoxicity of less than 1%, while on average in women aged ≥50 in Europe the yearly incidence of heart failure ranges between 0.2% to 2.2% [20], is considered acceptable. This would result in the following recommendations. First, for patients with a baseline LVEF ≥60%, without cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline and who do not smoke, further serial cardiac monitoring during trastuzumab treatment could be omitted. Since the cumulative incidence of severe cardiotoxicity in these patients after 4 years trastuzumab treatment is low (3.1%). Second, for patients with ≥1 risk factor, cardiac monitoring during the first 3 years would be recommended as the yearly increase and absolute numbers of non-

severe + severe cardiotoxicity were low after 3 years. Thereafter, cardiac monitoring could be performed in case of cardiac symptoms. The high reversibility rates of cardiotoxicity in a substantial number of patients with MBC, even after continuing trastuzumab, supports our proposed individualized LVEF monitoring scheme during (long-term) trastuzumab treatment.

We did not have sufficient data on the use or start of cardio-protective medication which could be of value in determining trastuzumab continuation after cardiotoxicity and in evaluating the reversibility of cardiotoxicity. Furthermore, we could not assess whether the use or start of cardio-protective medication could be helpful for the proposed individualized LVEF monitoring schemes. Medication including ACE-inhibitors (perindopril, lisinopril), beta-blockers (carvedilol, bisoprolol) or angiotensin receptor blocker (candesartan), has shown to attenuate LVEF declines and thereby potentially increase the reversibility of LVEF declines.[21-25]

To the best of our knowledge, this study is the largest in number with the longest follow-up duration investigating the reversibility of cardiotoxicity during trastuzumab in patients with HER2-positive MBC. Although the historical observational design of this cohort study provided a valuable opportunity to investigate clinical practice, some limitations inherent to historic cohorts should be mentioned. First, not all variables could retrospectively be collected and therefore not all variables, for example medication use and total dose of anthracycline treatment (mg/m^2) and radiation treatment (Gy), could be investigated. Although some missing data was observed for other variables as well, these variables could be used after multiple imputation as sensitivity analysis with complete case analysis showed similar risk factors associated with non-severe + severe cardiotoxicity as after multiple imputations (Supplementary Table S3). Second, due to the lack of clear cardiac monitoring guidelines, the timing of the LVEF measurements was not standardized but chosen by the treating physician. Therefore, ascertainment of cardiotoxicity could be delayed and detection bias cannot be excluded. However, information bias cannot have influenced the incidence of cardiotoxicity as LVEF measurements after cardiotoxicity were not taken into account. Third, most LVEFs (83%) were measured by MUGA scanning with a known high inter-observer and intra-observer variability in measuring the LVEF.[26, 27] However, by using a LMM analysis that takes into account all available LVEF measurements, the effect of this variability is minimized. Fourth, in estimating the cumulative incidence of cardiotoxicity per number of relevant risk factors, the effect size of the individual risk factors was not taken into account. Fifth, as the primary endpoint of the study was the development of cardiotoxicity, we did not investigate other cardiac co-morbidities that could develop over time as the median follow-up of the study was 15 months. Last, only 26 patients (6%) in our cohort received

dual HER2-targeted therapy, which is the current standard of first-line treatment for patients with HER2-positive MBC. However, as pertuzumab does not increases the risk of cardiotoxicity [28], the results of this study are likely to be applicable to daily clinical practice.

Conclusion

The cumulative incidence of severe cardiotoxicity in non-smoking patients with a baseline LVEF ≥60%, who had no cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline after 4 years trastuzumab treatment is low (3.1%). Therefore, serial cardiac monitoring can be omitted in these patients during long-term trastuzumab use.

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SUPPLEMENTAL MATERIAL
CHAPTER 2

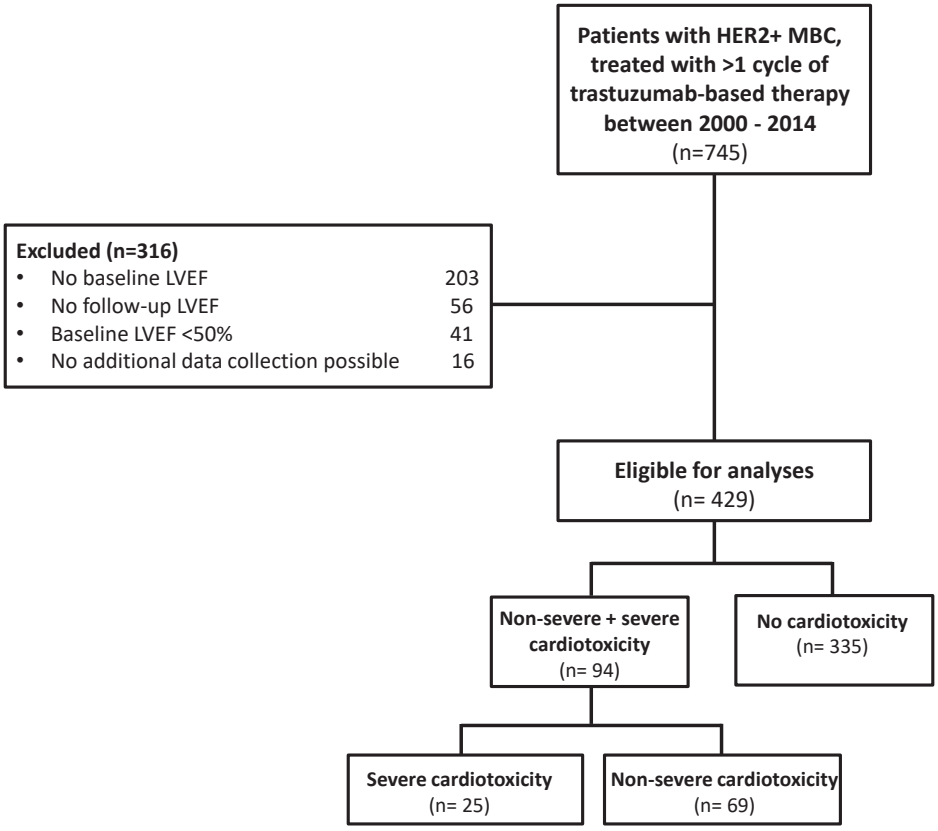


Figure S1. Flowchart of included patients

Abbreviations: HER2+, Human Epidermal growth factor Receptor 2 positive; MBC, metastatic breast cancer; LVEF, left ventricle ejection fraction.

Table S1. Differences in risk factors present at start of trastuzumab for MBC between included and excluded patients

Age (years)	
BMI (kg/m^2)	
<25	
25 – 30	
>30	
Hypertension	
Diabetes mellitus	
Smoking	
History of cardiac disease	
Baseline LVEF(%)[a]	
≥60	
<60	
Prior neoadjuvant/adjuvant trastuzumab	
Cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracyclines	
Cumulative anthracycline exposure (total number of courses)	
Adjuvant radiotherapy	
No	
Left side	
Right side	
Side unknown	
De novo MBC	

Abbreviations: BMI, body mass index; LVEF, left ventricle ejection fraction.

[a] 80 baseline LVEF were measured in the excluded patients.

[b] A course consist of doxorubicin 60 mg/m^2 or epirubicin 100 mg/m^2.

Included patients n=429 (%), median [IQR]	**Excluded patients n=339** (%), median [IQR]	**P-value**
54 [46-61]	53 [45-61]	0.699
		0.252
164 (38)	77 (23)	
130 (30)	73 (22)	
44 (10)	32 (9)	
98 (23)	60 (18)	0.928
28 (7)	19 (6)	0.637
90 (12)	53 (16)	0.502
35 (8)	26 (8)	0.373
		0.297
198 (46)	42 (53)	
231 (54)	38 (48)	
84 (20)	47 (14)	0.038
15 (3)	18 (5)	0.283
0 [0-6]	3 [0-6]	0.646
		0.084
171 (40)	152 (45)	
126 (29)	81 (24)	
100 (23)	68 (20)	
32 (7)	37 (11)	
118 (28)	86 (25)	0.506

Table S2. Risk factors present at start of trastuzumab for MBC associated with non-severe + severe cardiotoxicity and severe cardiotoxicity when adjusted for death

Age (years)

BMI (kg/m^2)
  <25
  25-30
  >30

Smoking
Baseline LVEF (%)   ≥60   <60
Prior neoadjuvant/adjuvant trastuzumab
Cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline
Cumulative anthracycline exposure (total number of courses)[a]
De novo metastatic breast cancer

Abbreviations: HR, hazard ratio; CI, confidence interval; BMI, body mass index; LVEF, left ventricle ejection fraction; MBC, metastatic breast cancer; REF, reference category.
Multivariable cause-specific Cox proportional hazard regression analysis with multiple imputations with substantive model compatible version of fully conditional specification (SMC-FCS) of

	Non-severe + severe cardiotoxicity			Severe cardiotoxicity		
	Adjusted HR	95% CI	P-value	Adjusted HR	95% CI	P-value
	1.01	0.99 – 1.03	0.235	NA		
	REF					
	1.24	0.71 – 2.15	0.447	NA		
	1.85	0.96 – 3.58	0.066	NA		
	2.12	1.21 – 3.72	0.008	2.14	1.23 – 3.73	0.007
	REF					
	1.44	0.90 – 2.29	0.125	1.43	0.91 – 2.24	0.122
	0.38	0.16 – 0.92	0.030	NA		
	5.37	1.30 – 22.12	0.017	2.93	1.18 – 7.30	0.019
	1.05	0.96 – 1.15	0.263	1.03	0.95 – 1.12	0.505
	0.85	0.50 – 1.47	0.571	NA		

diabetes mellitus, hypertension, smoking, history of cardiac disease, local radiotherapy of the breast and cardiotoxicity during prior neoadjuvant or adjuvant treatment with trastuzumab and/or anthracycline.

[a] A course consist of doxorubicin 60 mg/m^2 or epirubicin 100 mg/m^2.

Table S3. Sensitivity analysis with complete case analysis of risk factors present at start of trastuzumab treatment for MBC associated with non-severe + severe cardiotoxicity

Age (years)	
BMI (kg/m^2)	
<25	
25-30	
>30	
Hypertension	
Diabetes mellitus	
Smoking	
History of cardiac disease	
Baseline LVEF (%)	
≥60	
<60	
Prior neoadjuvant/adjuvant trastuzumab[b]	
Cardiotoxicity during prior neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline	
Cumulative anthracycline exposure (total number of courses)[b]	
Adjuvant radiotherapy	
No	
Left side	
Right side	
Side unknown	
De novo metastatic breast cancer	

Abbreviations: PH, proportional hazards; HR, hazard ratio; CI, confidence interval; BMI, body mass index; LVEF, left ventricle ejection fraction; MBC, metastatic breast cancer; REF, reference category.

	Univariable Cox PH[a]			Multivariable Cox PH[a]	
HR	95% CI	P-value	Adjusted HR	95% CI	P-value
1.01	0.99 – 1.02	0.579	1.01	0.98 – 1.04	0.574
REF					
1.07	0.63 – 1.81	0.810	0.80	0.38 – 1.68	0.547
1.79	0.94 – 3.42	0.078	2.00	0.88 – 4.53	0.096
1.21	0.77 – 1.92	0.414			
1.11	0.49 – 2.56	0.790			
1.88	1.13 – 3.16	0.013	2.32	1.20 – 4.47	0.012
1.34	0.69 – 2.58	0.389			
REF					
1.60	1.05 – 2.44	0.031	2.06	1.01 – 4.18	0.046
0.60	0.33 – 1.10	0.098	0.89	0.32 – 2.43	0.814
3.60	1.65 – 7.68	0.001	6.76	2.10 – 21.73	0.001
1.06	0.98 – 1.14	0.157	1.09	0.95 – 1.24	0.216
REF					
1.01	0.62 – 1.65	0.975			
1.00	0.57 – 1.73	0.986			
1.22	0.57 – 2.62	0.613			
1.28	0.79 – 2.06	0.315	0.91	0.41 – 2.02	0.815

[a] Not based on multiple imputations of diabetes mellitus, hypertension, smoking, history of cardiac disease, local radiotherapy of the breast and prior cardiotoxicity during treatment with trastuzumab or anthracyclines but based on only the complete cases.

[b] A course consist of doxorubicin 60 mg/m^2 or epirubicin 100 mg/m^2.

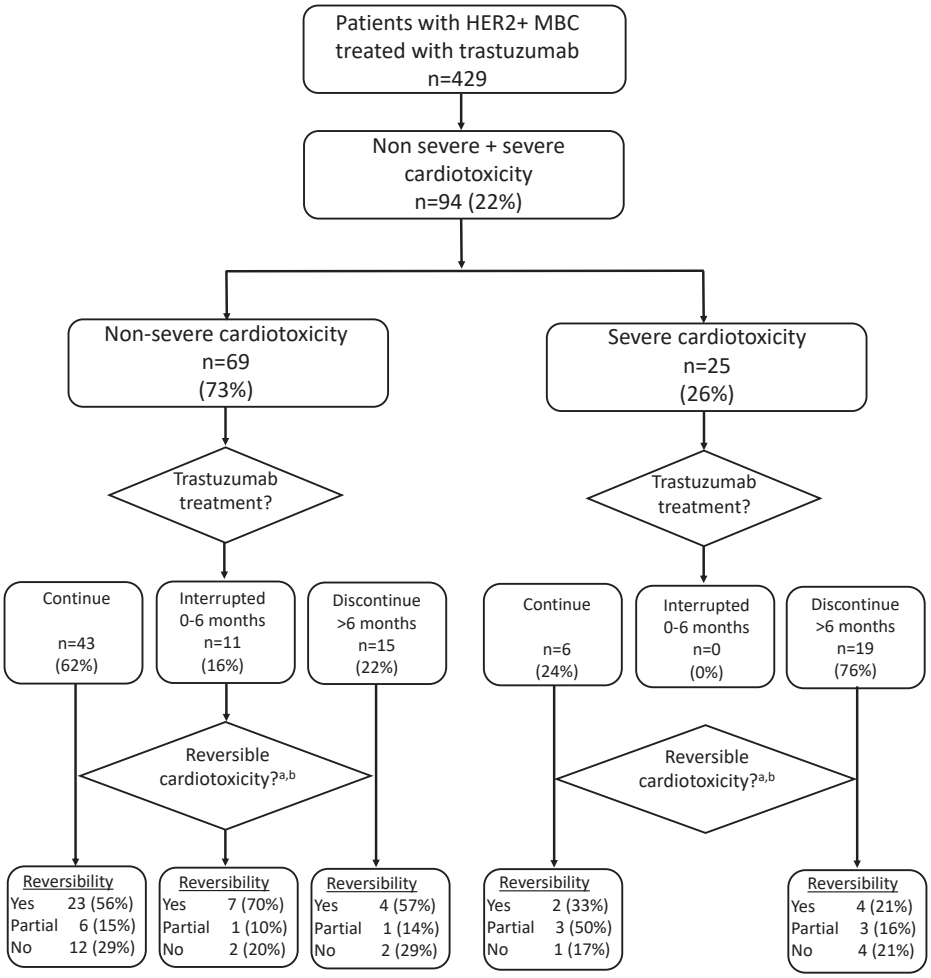
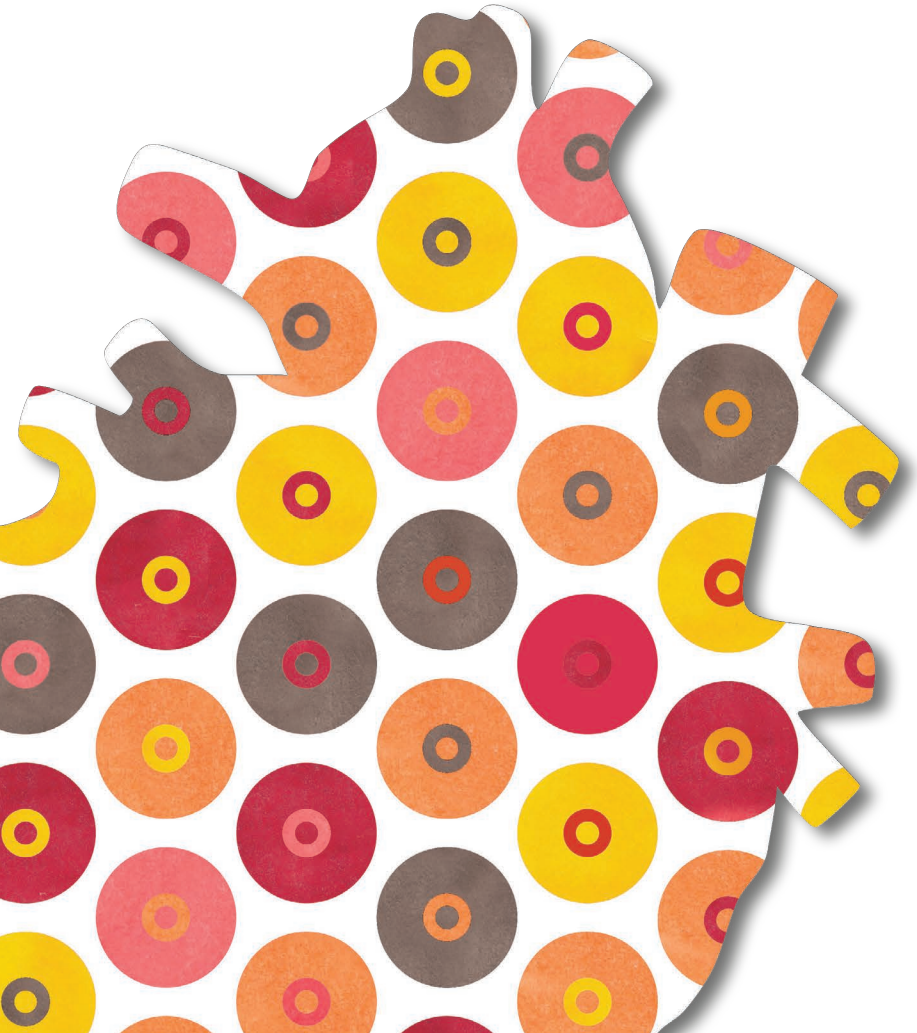


Figure S2. Reversibility of cardiotoxicity subdivided to the physicians' decision of trastuzumab (dis)continuation

Abbreviations: HER2+, Human Epidermal growth factor Receptor 2 positive; MBC, metastatic breast cancer.

[a] After development of cardiotoxicity, no LVEF measurements were available for some patients. Therefore, the reversibility of cardiotoxicity could not be assessed for every patient and the percentage is taken from the patients that received cardiac monitoring.

[b] Reversibility of non-severe and severe cardiotoxicity was defined as any LVEF increase to a value <5%-points below baseline value, partially reversibility as any LVEF increase ≥10-points from nadir and to a value >5%-points below baseline value and irreversibility as an absolute LVEF increase <10%-points from nadir and to a value >5%-points below baseline value.[14]



CHAPTER 3

Cardiac function in patients with HER2-positive metastatic breast cancer who start trastuzumab despite a left ventricular ejection fraction <50%

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ABSTRACT

Objective

We investigated the effect of trastuzumab on cardiac function in a real-world historic cohort of patients with HER2-positive metastatic breast cancer (MBC) with reduced baseline left ventricular ejection fraction (LVEF).

Results

Thirty-seven patients with HER2-positive MBC and baseline LVEF of 40-49% were included. Median LVEF was 46% (interquartile range [IQR] 44-48%) and median follow-up was 18 months (IQR 9-34 months). During this period, the LVEF did not worsen in 24/37 (65%) patients, while 13/37 (35%) patients developed severe cardiotoxicity defined as LVEF <40% with median time to severe cardiotoxicity of 7 months (IQR 4-10 months) after beginning trastuzumab. Severe cardiotoxicity was reversible (defined as LVEF increase to a value <5%-points below baseline value) in 7/13 (54%) patients, partly reversible (defined as absolute LVEF increase ≥10%-points from nadir to a value >5%-points below baseline) in 3/13 (23%) patients and irreversible (defined as absolute LVEF increase <10%-points from nadir and to a value >5%-points below baseline) in 3/13 (23%) patients. Likelihood of reversibility was numerically higher in patients who received cardio-protective medications (CPM), including ACE-inhibitors, beta-blockers and angiotensine-2 inhibitors, compared to those who did not receive any CPM (71% versus 13%, p=0.091)

Conclusion

Sixty-five percent of patients who received trastuzumab for HER2-positive MBC did not develop severe cardiotoxicity during a median follow-up of 18 months, despite having a compromised baseline LVEF. If severe cardiotoxicity occurred, it was at least partly reversible in more than two thirds of the cases. Risks and benefits of trastuzumab use should be balanced carefully in this vulnerable population.

INTRODUCTION

Trastuzumab has revolutionized the treatment of HER2-positive metastatic breast cancer (MBC). In conjunction with chemotherapy, objective response rates are high and long-term survival is observed in a subset of patients.[1-4] Cardiotoxicity, however, is a well-known side effect, and the reason why trastuzumab is contra-indicated in patients with reduced left ventricular ejection fraction (LVEF <50%) at baseline.[5, 6] In the absence of symptomatic cardiac dysfunction however, it is possible that the benefits of trastuzumab may outweigh the risk of severe cardiotoxicity.

Two studies have investigated trastuzumab initiation in patients with baseline LVEF <50%.[7, 8] An observational cohort study in 20 patients with early breast cancer (EBC) and LVEF <50% at baseline found that these patients more often developed symptomatic heart failure compared to those with baseline LVEF ≥50% (25% versus 4%).[8] In a clinical trial, 3 out of 30 patients (10%) with HER2-positive breast cancer (of which 58% were diagnosed with EBC and 42% with MBC) with an initial asymptomatic LVEF of 40-49% who received trastuzumab, developed LVEF decline of >10%-points from baseline or LVEF ≤35% despite cardio-protective medications (CPM) carvedilol and renin-angiotensin inhibitors.[7] Long-term continuation of trastuzumab in patients with HER2-positive MBC and compromised baseline LVEF has not been investigated extensively in these studies, as maximum follow-up was only 12 months [7] and only 42% of the included patients had MBC.[8] Additionally, the effect of trastuzumab initiation without appropriate CPM remains unknown. The aim of our historic real-world cohort study was to investigate the cardiac function of patients with HER2-positive MBC who received trastuzumab despite having baseline LVEF of 40-49%. In addition, the effect of CPM on the cardiac function was explored.

METHODS

Patients and data collection

Patients diagnosed with HER2-positive MBC between January 2000 and December 2014 receiving >1 cycle of trastuzumab-based treatment for advanced disease in one of eight participating Dutch hospitals were potentially eligible for this study. As previously described [6], patients were included if they received >1 cycle of trastuzumab, had baseline LVEF ≥40% or <50% within 30 days before the first trastuzumab administration, follow-up LVEF measurements during trastuzumab treatment, and complete clinical and medication data in the electronic medical records. Trained investigators systematically

Primary endpoints and definitions

Median follow-up was calculated from start of trastuzumab for MBC until last LVEF measurement or last trastuzumab dose, whichever came first. Interruption of trastuzumab was defined as trastuzumab discontinuation <6 months. Definitive discontinuation of trastuzumab was defined as trastuzumab discontinuation ≥6 months.

Severe cardiotoxicity was defined as LVEF <40% as per European Society of Medical Oncology (ESMO) guidelines which also do not specify a standardized interval for LVEF monitoring in the metastatic setting. [9] Reversibility of cardiotoxicity was defined as any LVEF increase to a value <5%-points below baseline value, partial reversibility as any absolute LVEF increase ≥10%-points from nadir and to a value >5%-points below baseline value, and irreversibility as any absolute LVEF increase <10%-points from nadir and to a value >5%-points below baseline value.[10] Use of CPM, including ACE-inhibitors, beta-blockers or angiotensine-2 inhibitors, was categorized into no CPM, primary CPM defined as prescription <30 days before trastuzumab initiation or secondary CPM defined as CPM prescription >1 week after start of trastuzumab.

Statistical analyses

Categorical variables are presented as numbers and percentages. Continuous variables with a non-normal distribution are presented as medians with interquartile range (IQR), and continuous variables with a normal distribution as means with standard deviations. Normality of continuous variables was evaluated by Shapiro-Wilk tests.

The characteristics of patients without CPM, with primary CPM and secondary CPM were compared using chi-square test for categorical variables and analysis of variance (ANOVA) test for continuous variables. Additionally, a log-rank test was used to compare the rate of reversibility between these groups. Overall survival, defined as time from diagnosis of MBC until death from any cause or last follow-up, was calculated using Kaplan-Meier survival estimates. Data analyses were performed using SPSS (version 26.0).

RESULTS

Patient characteristics

From a real-world cohort of 745 patients who received trastuzumab for HER2-positive MBC, 37 patients with a median LVEF of 46% (IQR 44-48%) met the eligibility criteria (Supplementary Figure S1). Patient and treatment characteristics are shown in Table 1. Median frequency of LVEF monitoring was 4 times annually (IQR 2 – 6). Included

patients differed from excluded patients in shorter duration of neoadjuvant/adjuvant trastuzumab, previous cardiotoxicity during neoadjuvant/adjuvant treatment, history of cardiac disease and hypertension (Table 1).

Severe cardiotoxicity and reversibility

Over a median follow-up of 18 months (IQR 9-34 months) and median duration of trastuzumab exposure of 14 months (IQR 8-28 months), 13 patients (35%) developed severe cardiotoxicity at a median time on trastuzumab of 7 months (IQR 4-10 months, Supplementary Figure S2). Reversibility of cardiotoxicity and trastuzumab disposition is depicted in Figure 1. In 1 out of 2 patients (50%) who interrupted trastuzumab due to cardiotoxicity, trastuzumab was reintroduced for another 8 months. The development of cardiotoxicity was the cause of discontinuation in all seven patients who discontinued trastuzumab treatment. Median overall survival in all patients was 47 months (IQR 30-65 months).

Cardiac medication

In total, 11 patients (30%) received primary CPM consisting of a beta-blocker (n=4), ACE-inhibitor (n=3) or both (n=4) with 10 patients (27%) receiving secondary CPM with a beta-blocker (n=2), ACE-inhibitors (n=4) or both (n=4). Reversible cardiotoxicity was observed more often in patients with CPM versus without CPM (71% versus 13% respectively, log-rank p-value=0.091). Median nadir LVEF with primary CPM did not differ from those without CPM (LVEF 42% versus 46%, p=0.437, Table 2). Cardiotoxicity was reversible in 3 of 4 patients (75%) receiving secondary CPM with trastuzumab interruption and in 2 of 3 patients (67%) continuing trastuzumab with secondary CPM (Supplementary Figure S2). One patient receiving secondary CPM and continuing trastuzumab had partly reversible cardiotoxicity. No difference was observed between median nadir LVEF of patients with at least partly reversible or irreversible cardiotoxicity (LVEF 35% versus 39%, p=0.398).

Table 1. Difference in baseline characteristics between excluded and included patients

Clinical and treatment characteristics
Age (years)[a]
Stage at disease presentation Metachronous MBC De novo MBC
ER status[b] Positive Negative
Neoadjuvant/adjuvant therapy No Anthracycline with trastuzumab Anthracycline without trastuzumab Other
Duration of neo-adjuvant/adjuvant trastuzumab (months)
Cumulative anthracycline exposure (courses)[b]
Radiotherapy of the breast No Left side Right side
Previous cardiotoxicity during neoadjuvant/adjuvant treatment with trastuzumab and/or anthracycline No Yes No neo-adjuvant/adjuvant treatment
Duration of trastuzumab administration for MBC (months)
First-line treatment Trastuzumab + taxanes Trastuzumab + capecitabine Trastuzumab + vinorelbine Trastuzumab + pertuzumab + CT Trastuzumab + endocrine Trastuzumab monotherapy Other
Median overall survival (months)
LVEF (%)[a] ≥50% 45-49% 40-44%

	Included patients n=37 No. (%), median [IQR]	Excluded patients n=708 No. (%), median [IQR]	P-value
	52 [44-62]	54 [46-61]	0.457
			0.254
	30 (81)	525 (74)	
	7 (18)	183 (26)	
			0.981
	21 (57)	393 (56)	
	16 (43)	315 (44)	
			0.327
	16 (43)	369 (52)	
	5 (31)	203 (29)	
	15 (41)	99 (14)	
	1 (3)	37 (5)	
	8 [5-12]	12 [12-12]	0.021
	6 [0-6]	3 [0-6]	0.107
			0.339
	16 (43)	302 (43)	
	13 (35)	190 (37)	
	8 (22)	159 (22)	
			<0.001
	12 (32)	316 (45)	
	8 (22)	23 (3)	
	17 (46)	369 (52)	
	14 [8-28]	15 [6-36]	0.152
			0.729
	16 (43)	238 (34)	
	0 (0)	20 (3)	
	3 (8)	89 (13)	
	1 (3)	20 (3)	
	7 (19)	30 (4)	
	0 (0)	15 (2)	
	10 (27)	296 (42)	
	47 [30-65]	38 [20-71]	0.404
			<0.001
	0 (0)	505 (71)	
	26 (63)	0 (0)	
	11 (27)	0 (0)	

Table 1. Continued

Clinical and treatment characteristics	
Cardio-protective medications use	
None	
Before start of trastuzumab treatment	
During trastuzumab treatment	
Imaging modalities used for LVEF measurement	
MUGA scan	
Echocardiography	
MUGA scan + echocardiography	
BMI (kg/m^2)[a]	
<25	
25-30	
>30	
History of cardiac disease[a]	
Diabetes mellitus[a]	
Hypertension[a]	
Hypercholesterolemia[a]	
Smoking[a]	
Current	
Former	
No	

Abbreviations: BMI, body mass index; ER, estrogen receptor; LVEF, left ventricle ejection fraction; MBC, metastatic breast cancer; MUGA, multigated acquisition scan; NA, not applicable.

	Included patients n=37 No. (%), median [IQR]	Excluded patients n=708 No. (%), median [IQR]	P-value
			NA
	15 (41)	NA	
	11 (30)	NA	
	11 (30)	NA	
			0.299
	28 (76)	537 (76)	
	2 (5)	23 (3)	
	7 (19)	52 (7)	
			0.780
	14 (38)	225 (31)	
	9 (24)	193 (27)	
	6 (16)	68 (10)	
	10 (27)	49 (7)	<0.001
	3 (8)	42 (6)	0.721
	18 (43)	131 (19)	0.002
	3 (8)	50 (7)	0.690
			0.522
	5 (14)	70 (10)	
	6 (16)	60 (8)	
	17 (46)	283 (40)	

[a] At start of trastuzumab treatment for MBC.

[b] Estrogen receptor positivity was defined as ≥ 10% positive nuclear staining based on the Dutch guideline.

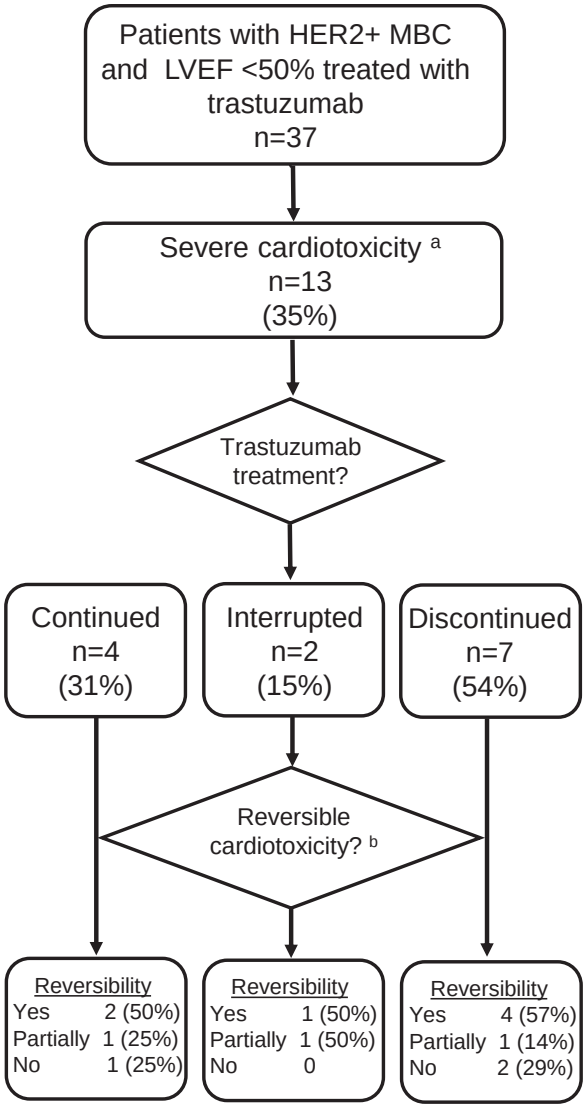

Figure 1. Reversibility of cardiotoxicity subdivided for the physicians' decision of trastuzumab (dis)continuation

Abbreviations: HER2+, Human Epidermal growth factor Receptor 2 positive; MBC, metastatic breast cancer.

[a] Severe cardiotoxicity was defined as absolute LVEF <40%.

[b] Reversibility of severe cardiotoxicity was defined as any LVEF increase to a value <5%-points below baseline value, partially reversibility as any LVEF increase ≥10-points from nadir and to a value >5%-points below baseline value and irreversibility as an absolute LVEF increase <10%-points from nadir and to a value >5%-points below baseline value.

Table 2. Clinical characteristics of patients without CPM, primary CPM or secondary CPM

Clinical and treatment characteristics	All patients (n=37)	Patients without CPM (n=16)	Patients with primary CPM (n=11)	Patients with secondary CPM (n=10)
Severe cardiotoxicity[a], n (%)	13 (35)	2 (13)	3 (27)	8 (80)
Time to cardiotoxicity, months [IQR]	7 [4-10]	8 [7-NA]	5 [3-NA]	12 [4-26]
Reversibility[b], n (%)				
No	3 (8)	1 (6)	0 (0)	2 (20)
Partial	7 (19)	0 (0)	4 (36)	1 (10)
Yes	27 (73)	2 (13)	7 (64)	8 (80)
Trastuzumab treatment, n (%)				
Continued	23 (62)	8 (03)	7 (64)	8 (80)
Interrupted	6 (16)	4 (25)	2 (18)	1 (10)
Definitive discontinued	7 (19)	3 (19)	2 (18)	2 (20)
LVEF, median % (IQR)				
Baseline	46 [44-48]	48 [46-49]	46 [43-48]	44 [41-46]
Nadir	42 [38-46]	46 [43-47]	42 [32-44]	38 [29-41]
Highest	54 [50-58]	59 [53-65]	51 [50-57]	52 [50-56]

Abbreviations: LVEF, left ventricular ejection fraction; IQR, interquartile range.

[a] Severe cardiotoxicity was defined as LVEF <40%.

[b] Reversibility was defined as any LVEF increase to a value <5% below baseline value, partial reversibility as any absolute LVEF increase ≥10% from nadir and to a value >5% below baseline value, and irreversibility as any absolute LVEF increase <10% from nadir and to a value >5% below baseline value.

DISCUSSION

The current study reports on the cardiac status of a real-world cohort of patients with HER2-positive MBC and baseline LVEF of 40-49% who received trastuzumab-containing systemic therapy. According to current Food and Drug Administration (FDA) and European Medicines Agency (EMA) recommendations, trastuzumab treatment was not recommended for the 37 patients (5% of all patients with HER2-positive MBC) included in our study due to impaired LVEF. These patients received trastuzumab over a median duration of 14 months with an overall survival of 47 months which is comparable to patients with HER2-positive MBC who received trastuzumab with LVEF ≥50% at baseline and to the excluded patients (Table 1).[6]

We showed that 65% of patients with HER2-positive MBC and an impaired baseline LVEF did not develop severe cardiotoxicity during trastuzumab treatment. Moreover, if severe cardiotoxicity occurred, it was at least partly reversible in more than two thirds

of the cases. Similar to previous reports [7], we observed an effect of CPM on the LVEF reversibility, although it was not statistically significant likely due to the underpowered small sample size. Larger, randomized studies with a longer follow-up time are warranted to further investigate whether optimal CPM can lead to trastuzumab being safely administered in this vulnerable population. For now, the risks and benefits of trastuzumab in patients with HER2-positive MBC and an impaired baseline LVEF must be balanced carefully in close collaboration with a cardiologist and in a shared-decision making context to obtain optimal patient-centered outcomes.

An important limitation of the current study is that most LVEFs (78%) were measured with MUGA scans which have large inter- and intra-observer variations [12] possibly explaining why physicians are more likely to continue trastuzumab in asymptomatic patients with LVEFs below 50%. Second, our definition of cardiotoxicity reversibility remains relative as it is defined as LVEF increase to baseline value, which is still compromised in this population. Third, due to the small sample size (n=37), our study is underpowered to assess the impact of CPM on cardiac function during trastuzumab therapy and its effect on reversibility of LVEF declines. Fourth, we were also unable to capture clinical symptomatology to further delineate the impact of trastuzumab on cardiac function in our study population. Finally, only 3 patients (8%) received dual HER2-targeted therapy including pertuzumab which is the current standard treatment for patients with HER2-positive MBC. However, as pertuzumab does not increase the risk of cardiotoxicity [13], the results of this study are likely to be applicable to current routine clinical practice. Despite these limitations, we were able to investigate the cardiac function after initiating trastuzumab in patients with HER2-positive MBC and LVEF of 40-49% during a median follow-up of 18 months, which provides insight for further prospective research.

Conclusion

Despite having an impaired baseline LVEF, 65% of patients who received trastuzumab for HER2-positive MBC did not develop severe cardiotoxicity during a median follow-up of 18 months. If severe cardiotoxicity occurs, it was at least partly reversible in about two thirds of the cases. Risks and benefits of trastuzumab in this vulnerable population should be balanced carefully.

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SUPPLEMENTAL MATERIAL
CHAPTER 3

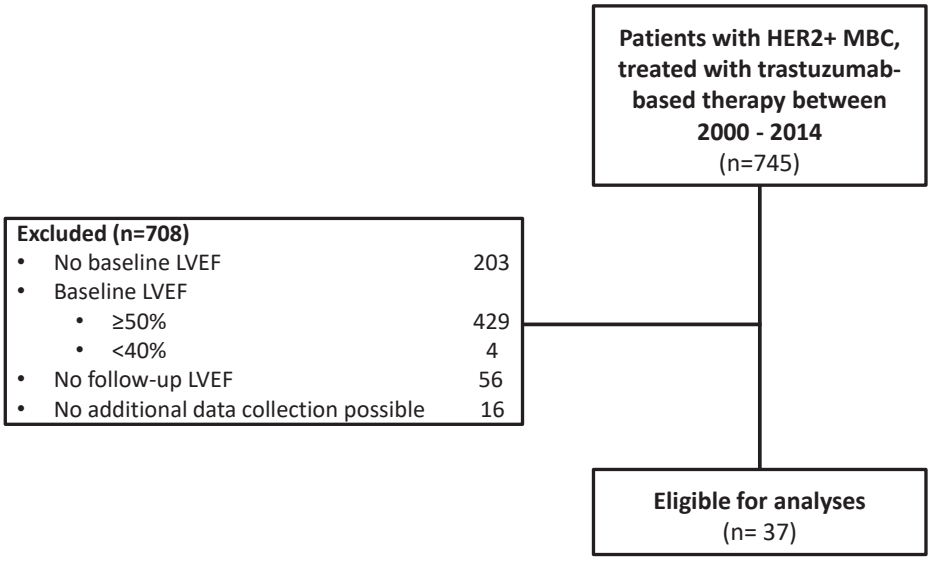


Figure S1. Flowchart of included patients

Abbreviations: HER2+, Human Epidermal growth factor Receptor 2 positive; LVEF, left ventricle ejection fraction; MBC, metastatic breast cancer.

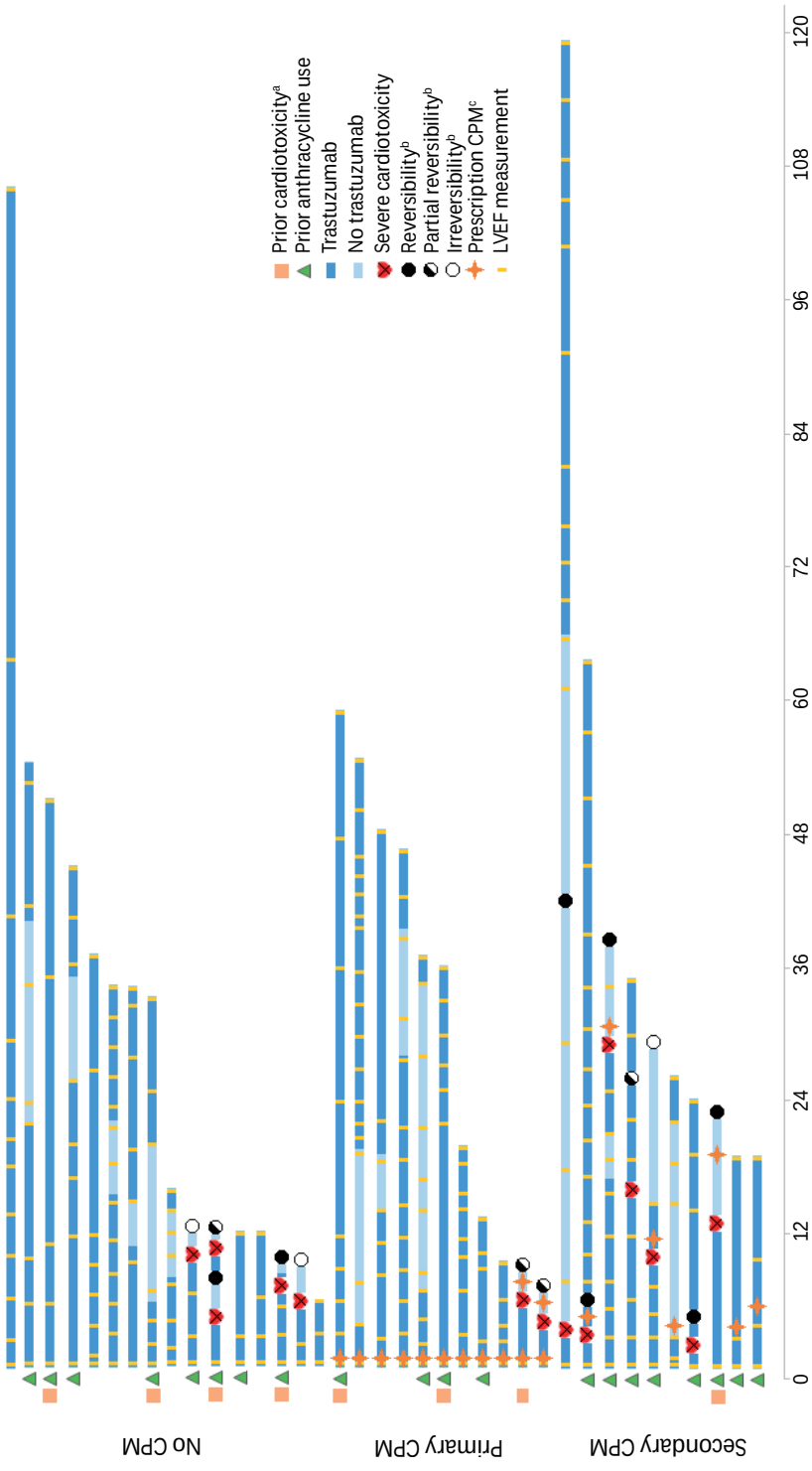

Prior cardiotoxicity[a]
Prior anthracycline use
Trastuzumab
No trastuzumab
Severe cardiotoxicity
Reversibility[b]
Partial reversibility[b]
Irreversibility[b]
Prescription CPM[c]
LVEF measurement
No CPM
Primary CPM
Secondary CPM
0
12
24
36
48
60
72
84
96
108
120
Months from start trastuzumab treatment to last LVEF or trastuzumab discontinuation[d]

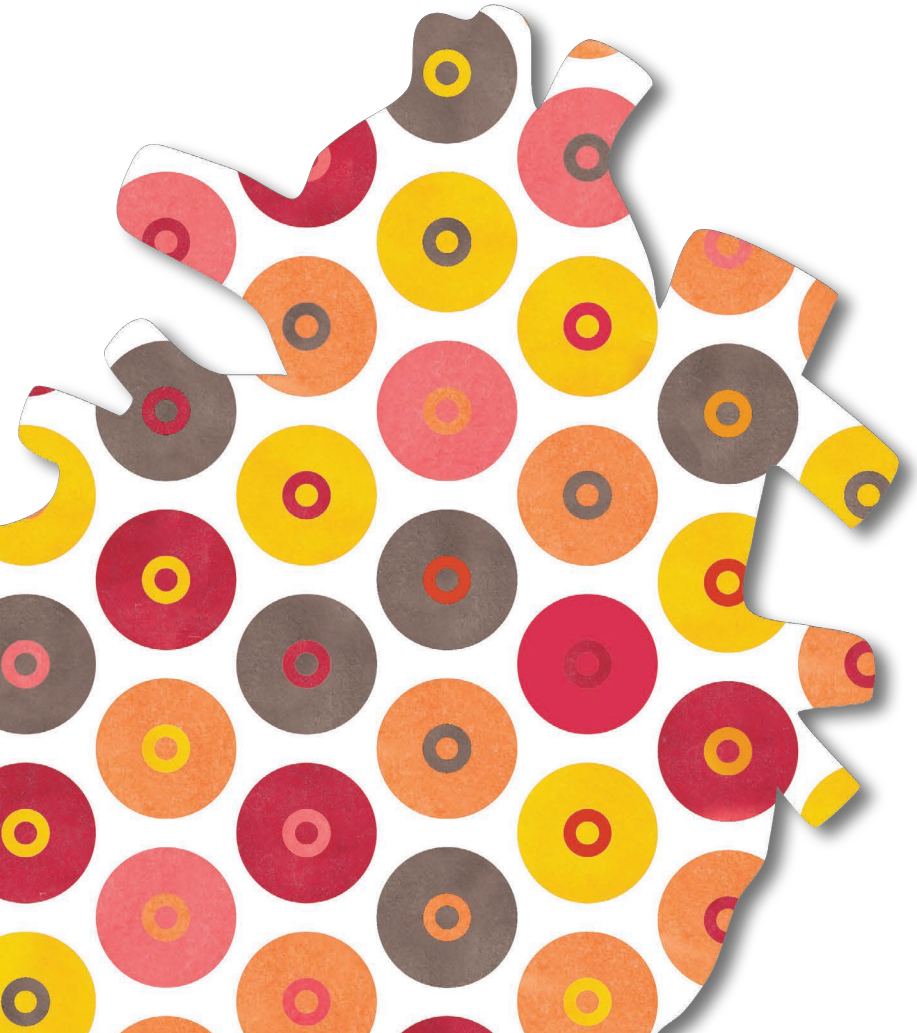
Figure S2. Swimmersplot of trastuzumab course in all patients with LVEF <50%

[a] Prior cardiotoxicity was defined as cardiotoxicity defined as LVEF >10%-points to a LVEF <50% during prior treatment with trastuzumab and/or anthracycline.

[b] Reversibility of severe cardiotoxicity was defined as any LVEF increase to a value <5%-points below baseline value, partially reversibility as any LVEF increase ≥10-points from nadir and to a value >5%-points below baseline value and irreversibility as an absolute LVEF increase <10%-points from nadir and to a value >5%-points below baseline value.

[c] For 3 patients who received cardio-protective medication during trastuzumab, the exact timing of prescription was unknown.

[d] Whichever occurred last.



CHAPTER 4

Radiological complete remission in HER2-positive metastatic breast cancer patients: what to do with trastuzumab?

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Breast Cancer Res Treat. 2019 Dec; 178(3)

ABSTRACT

Objective

Patients with HER2-positive metastatic breast cancer (MBC) treated with trastuzumab may experience durable tumor response for many years. It is unknown if patients with durable radiological complete remission (rCR) can safely discontinue trastuzumab. We analyzed clinical characteristics associated with rCR and overall survival (OS) in a historic cohort of patients with HER2-positive MBC and studied the effect of stopping trastuzumab in case of rCR.

Methods

We included patients with HER2-positive MBC treated with first or second line trastuzumab-based therapy in eight Dutch hospitals between 2000 and 2014. Data were collected from medical records. We used multivariable regression models to identify independent prognostic factors for rCR and OS. Time-to-progression after achieving rCR for patients who continued and stopped trastuzumab, and breast cancer-specific survival were also evaluated.

Results

We identified 717 patients with a median age of 53 years at MBC diagnosis. The median follow-up was 109 months (IQR 72 – 148). The strongest factor associated with OS was achievement of rCR, adjusted hazard ratio 0.27 (95% CI 0.18 – 0.40). RCR was observed in 72 patients (10%). Ten-year OS-estimate for patients who achieved rCR was 52 versus 7% for patients who did not achieve rCR. Thirty patients with rCR discontinued trastuzumab, of whom 20 (67%) are alive in ongoing remission after 78 months median follow-up since development of rCR. Of forty patients (58%) who continued trastuzumab since rCR, 13 (33%) are in ongoing remission after 68 months median follow-up. Median time-to-progression was 14 months.

Conclusions

Achieving rCR is the strongest predictor for improved survival in patients with HER2-positive MBC. Trastuzumab may be discontinued in selected patients with ongoing rCR. Further research is required to identify patients who have achieved rCR and in whom trastuzumab may safely be discontinued.

INTRODUCTION

Metastatic breast cancer (MBC) is considered an incurable disease, with median overall survival (OS) for patients averaging around 34 months.[1-3] Outcome for patients with HER2-positive MBC has dramatically improved since the introduction of trastuzumab.[2-6] Radiological complete remission (rCR) is not uncommon and patients may remain in complete remission for many years.[7-10] The addition of pertuzumab to first-line trastuzumab-based therapy in HER2-positive MBC has increased median OS to 56 months.[11] Other anti-HER2 drugs, including lapatinib and trastuzumab-emtansine (T-DM1), have extended treatment options for patients with HER2-positive MBC, and patients' survival can be prolonged even further.[5, 11-14]

In HER2-positive MBC patients on anti-HER2 therapy, various factors are associated with long OS such as young age (< 50 years) [15-17] , good performance status [9, 17, 18], de novo metastases [9, 17, 18], no previous treatment with trastuzumab [19-21], limited tumor load (i.e. oligo-metastases or single organ metastases) [9, 15, 22], and achievement of rCR [9, 18, 23, 24]. Most studies show an association with estrogen receptor (ER)-positivity and improved survival [4, 15, 17, 24], but not all [23, 25]. What remains unsure is whether additional local treatment of metastases improves survival. Moreover, oncologists question if and when trastuzumab can be stopped after achieving rCR upon trastuzumab-based therapy.[1, 26] A survey among 44 oncologists in Canada showed that after achieving clinical or radiological CR most oncologists continue trastuzumab therapy until progression (78%) or onset of toxicity (5%).[26] A couple of studies evaluated the effect of stopping trastuzumab in patients with HER2-positive MBC.[9, 19, 27] However, numbers were small [9], follow-up was rather short [19, 27] and not all patients had achieved CR upon trastuzumab-based therapy.[19]

Therefore, our aim was to evaluate which characteristics are associated with survival and achieving rCR in patients with HER2-positive MBC treated with trastuzumab-based therapy in a historic cohort with long follow-up. Additionally, we thoroughly describe characteristics of patients with rCR, long-term outcome of patients with rCR and the effect of trastuzumab discontinuation after achieving rCR.

METHODS

Patients and data collection

Patients were identified from the Netherlands Cancer Registry (NCR) and linked with the institutes' tumor registries from eight Dutch hospitals (two referral and six regional hospitals). We included all patients with histologically proven HER2-positive MBC who received more than one gift trastuzumab-based palliative therapy as first- or second line in one of these hospitals between January 2000 and December 2014.

Four clinicians (TS, NB, HR and CS) systematically extracted clinical characteristics from medical records using case record forms. Recorded data included date of birth, date of diagnosis of primary tumor, date of MBC, date of recurrence, tumor characteristics of the primary tumor, number and location of initial distant metastases, local ablative treatment (LAT) for metastases (surgery, radiotherapy, or radiofrequency ablation), systemic treatment for the primary tumor and for MBC, response to treatment, date of last follow-up and if death occurred the date and cause of death. The location of distant metastases was grouped into the following categories: lymph nodes (beyond locoregional), liver, lung, bone, central nervous system [(CNS); including leptomeningeal], skin and other. Oligo-MBC was defined as one to three detected metastases, not necessarily limited to a single organ. HER2-status of the primary tumor or MBC was determined according to the Dutch guideline [28]. HER2-positivity was defined as an immunohistochemistry score of 3+ or 2+ and amplification measured by in situ hybridization.[29] ER and progesterone receptor positivity was defined as ≥ 10% positive nuclear staining.[28, 30] The first chemotherapy or targeted therapy administered for MBC was defined as first-line therapy for MBC. The next therapy that was started following disease progression was defined as second-line therapy. Subsequent lines were counted in this manner. Progression was defined as either progression detected on radiological imaging assessed by local radiologist or clinical deterioration most likely caused by MBC. Radiologic response was assessed during routine follow-up by local radiologists. Radiologic complete response was defined as no-evidence-of-disease (NED) on radiological examination of the previously detected metastases (either contrast-enhanced computed tomography (CT), contrast-enhanced magnetic resonance imaging, or [18F]-fluorodeoxyglucose (FDG) positron emission tomography-CT) upon systemic treatment. Bone metastases were classified as rCR if metabolic activity normalized compared to start, seen on either FDG-PET-CT or bone scintigraphy. Patients who received successful LAT (i.e. NED after local treatment) for their metastases without rCR upon systemic treatment were not classified as having achieved rCR upon systemic therapy. Follow-up scans were performed at discretion of the treating physician in line with institutional guidelines.

Statistical analyses

The primary endpoint was OS, defined as date of diagnosis of MBC until death from any cause or last follow-up.[31] For patients last known to be alive, OS data were censored at the time of last follow-up visit. Breast cancer-specific survival (BCSS), defined as date of diagnosis of MBC until death from breast cancer or last follow-up, was a secondary outcome. Follow-up time was calculated with the reverse Kaplan-Meier method. Cox-proportional hazard models were used to identify prognostic factors for survival. Kaplan-Meier survival estimates were calculated for patients with and without the most important prognostic factor according to the Cox-model and compared with the log-rank test. We explored characteristics of patients who continued and patients who stopped trastuzumab after achievement of rCR. As date of radiological evaluation was not standardized, the time to achieve rCR that was recorded does not adequately represent the time actually needed to achieve rCR. We therefore used logistic regression rather than proportional hazard models to identify factors associated with higher odds of achieving rCR. Hazard ratios (HR) and odds ratios (OR) are given with their corresponding 95% confidence intervals (CI). Factors with a p-value < 0.10 for univariable associations with outcomes were included in the multivariable models. P-values < 0.05 were considered statistically significant; all tests were two-sided. Statistical analyses were performed using SPSS 25.0 (IBM Corp., Armonk, NY, USA). Figures were generated using Graph Pad Prism 5.0 (Graph Pad Software, La Jolla, CA, USA).

RESULTS

Patient characteristics

Seven hundred fifty-eight patients who received trastuzumab for MBC were identified and 717 patients were included in the final analysis. Reasons for exclusion were no trastuzumab as first- or second-line treatment for MBC (n = 21), only one cycle of trastuzumab received (n = 11), diagnosis of another cancer that determined prognosis (n = 4) and incompleteness of data (n = 5) (Supplementary Figure 1). Patient and tumor characteristics are shown in Table 1. Median age at diagnosis of MBC was 53 years (interquartile range [IQR] 46 – 61 years). One-hundred forty-three (20%) patients were diagnosed with oligo-MBC. Median time from breast cancer diagnosis until MBC was 26 months (IQR 0 – 56 months), and 194 patients (27%) were diagnosed with de novo metastases. In total, 595 patients (83%) had not received trastuzumab previously. Five-hundred fifteen patients (72%) received trastuzumab as first-line treatment for MBC.

Survival and predictive factors

After 109 months (IQR 72 – 148 months) of median follow-up, 579 patients had died, of whom 566 (98%) were due to MBC. The median OS for all patients was 37 months (IQR 21 – 72 months). Achievement of rCR had the strongest impact on death; adjusted HR of 0.27, 95% CI 0.18–0.40 (Table 2). Other factors significantly associated with longer OS in multivariable regression analysis were: longer interval from breast cancer diagnosis until MBC, ER-positivity, single-organ metastases, no neoadjuvant/adjuvant trastuzumab, de novo MBC, successful LAT of metastases. Liver, brain, and skin metastases showed a significant unfavorable association with OS. Analyses for BCSS were in line with OS data (data not shown).

Table 1. Baseline characteristics

Clinical and pathological characteristics	**All patients (n=717)**
Age at diagnosis MBC, years	
Median (IQR)	53 (46-61)
Time until MBC, months	
Median (IQR)	26 (0-56)
De novo MBC, no. (%)	
De novo MBC	194 (27)
Metachronous MBC	523 (73)
ER-status at diagnosis, no. (%)	
ER-positive	399 (56)
ER-negative	316 (44)
Single organ metastases, no. (%)	
Single organ metastases	329 (46)
More organs metastases	388 (54)
Oligo-metastases (≤ 3 metastases), no. (%)	
Oligo-metastases	143 (20)
Multiple metastases	562 (80)
Location of metastases at diagnosis MBC, no. (%)	
Bone	395 (55)
Liver	280 (39)
Lymph nodes	260 (36)
Lung	233 (33)
Skin	50 (7)
CNS	35 (5)
Prior neoadjuvant/adjuvant trastuzumab, no. (%)	
Yes	123 (17)
No	595(83)

Table 1. Continued

Clinical and pathological characteristics	All patients (n=717)
Moment first trastuzumab for MBC, no. (%)	
Trastuzumab received in 1st line	515 (72)
Trastuzumab received in 2nd line	202 (28)
First-line treatment, no. (%)	
Trastuzumab + taxanes	303 (42)
Trastuzumab + vinorelbine	91 (13)
Trastuzumab + capecitabine	24 (3)
Trastuzumab + other	29 (4)
Trastuzumab + endocrine therapy	30 (4)
Trastuzumab monotherapy	16 (2)
Trastuzumab + pertuzumab + CT	22 (3)
Successful local ablative treatment of metastases, no. (%)	
Yes	60 (9)
No	647 (91)
Achievement of rCR, no. (%)	
rCR achieved	72 (10)
rCR not achieved	645 (90)

Abbreviations: MBC, metastatic breast cancer; IQR, interquartile range; ER, estrogen receptor; CNS, central nervous system, rCR, radiological complete remission. % is based on known values.

Achievement of rCR

Seventy-two patients (10%) achieved rCR, of whom 42 (58%) are alive at last follow-up. The median OS for patients who achieved rCR was 142 months (11.8 years) (IQR 61-not reached) compared to median OS of 35 months (IQR 19 – 61 months) for patients who did not achieve rCR (p-value < 0.001, Figure 1). The 10-year OS estimate for patients who achieved rCR was 52% compared to 7% for patients who did not achieve rCR (Figure 1). Sixty-six patients achieved rCR upon treatment with trastuzumab combined with chemotherapy, four upon endocrine treatment combined with trastuzumab and two upon endocrine treatment only. Clinical characteristics at diagnosis of MBC independently associated with a higher chance to achieve rCR were oligo-metastases (adjusted odds ratio [aOR] 4.01, 95% CI 2.15 – 7.46), and de novo MBC (aOR 4.62, 95% CI 1.80 – 11.47). Patients who received trastuzumab as second-line treatment had significantly lower chance to achieve rCR (aOR 0.26, 95% CI 0.12 – 0.57, Table 3).

Table 2. Adjusted hazard ratios for death – multivariable Cox regression analyses

	Adjusted HR	95% CI	P-value
Time until MBC	0.99	0.99-0.99	0.001
ER-positive	0.70	0.59-0.84	< 0.001
Oligo-metastases (≤ 3 metastases)	0.85	0.64-1.12	0.242
Single-organ metastases	0.73	0.59-0.90	0.003
Liver metastases	1.34	1.10-1.62	0.003
Lung metastases	0.99	0.82-1.20	0.890
Skin metastases	1.59	1.16-2.16	0.003
CNS metastases	1.63	1.08-2.46	0.020
Prior neoadjuvant/adjuvant trastuzumab			
Prior trastuzumab	1		
No neoadjuvant/adjuvant trastuzumab	0.60	0.47-0.76	< 0.001
No prior trastuzumab - de novo MBC	0.42	0.31-0.57	< 0.001
Radiologic complete remission	0.27	0.18-0.40	< 0.001
Successful local ablative treatment of metastases	0.49	0.31-0.78	0.002

Abbreviations: HR, hazard ratio; CI, confidence interval; MBC, metastatic breast cancer; ER, estrogen receptor; rCR, radiological complete remission.
Multivariable Cox regression analysis, values are adjusted for all other variables. Selection of variables is based on statistically significant variables ($p < 0.10$) in univariable models.

Table 3. Adjusted odds ratios for achieving radiological complete remission

	Adjusted OR	95% CI	P-value
ER-positive	0.88	0.51-1.52	0.638
Oligo-metastases (≤ 3 metastases)	4.19	2.21-7.94	< 0.001
Single organ metastases	0.97	0.49-1.93	0.938
Bone metastases	0.42	0.23-0.77	0.005
Liver metastases	0.54	0.28-1.04	0.066
Lung metastases	0.55	0.28-1.08	0.084
Prior trastuzumab	1	-	-
No adjuvant trastuzumab	2.80	1.13-6.93	0.026
No prior trastuzumab - de novo MBC	5.14	2.01-13.18	0.001
Trastuzumab second line	0.26	0.12-0.57	0.001

Abbreviations: OR, odds ratio; CI, confidence interval; MBC, metastatic breast cancer; ER, estrogen receptor. Multivariable logistic regression analyses, values are adjusted for all other variables. Selection of variables is based on statistically significant variables ($p < 0.10$) in univariable models.

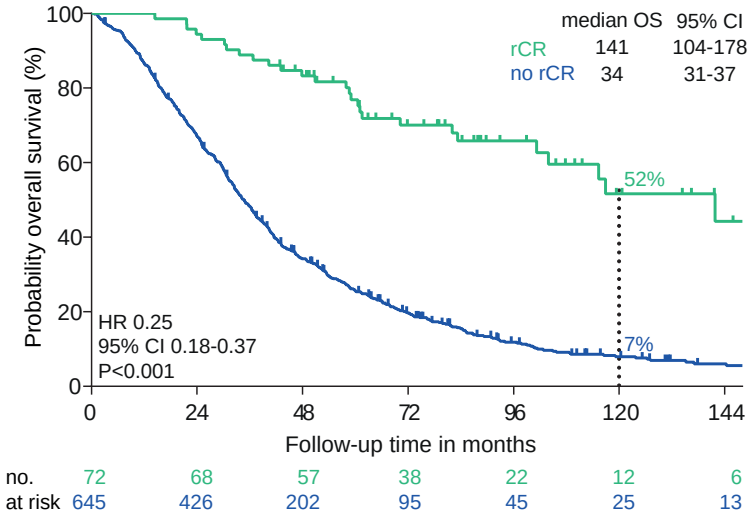

Figure 1. Probability of overall survival after diagnosis of metastatic breast cancer for patients who achieved radiologically complete remission and patients who did not

Abbreviations: rCR, radiological complete remission; OS, overall survival; HR, hazard ratio; CI, confidence interval.

The effect of discontinuation of trastuzumab after achieving rCR

Thirty patients (43%) discontinued trastuzumab after achieving rCR. The median time from onset of rCR to discontinuation was 6 months (IQR 0 – 9 months). Twenty patients (67%) who stopped trastuzumab after rCR remain in complete remission, with a median follow-up of 78 months (IQR 60 – 100 months) since onset of rCR. Eight patients (27%) who stopped trastuzumab after rCR experienced disease progression with a median time-to-progression (TTP) of 15 months (IQR 10 – 18 months) since last gift of trastuzumab. In five patients with disease progression after rCR trastuzumab was restarted. Four patients experienced stable disease for median time of 23 months since restart of trastuzumab. One of these patients achieved a new rCR upon trastuzumab-based therapy and is in ongoing remission at last follow-up with ongoing trastuzumab-based therapy (Figure 2). From the twenty patients who remain in rCR since discontinuation of trastuzumab, metastatic lesions were pathologically proven at diagnosis of MBC in eight.

Forty patients (58%) continued trastuzumab treatment after achieving rCR. Of them, 13 (33%) are in ongoing remission after 68 months (IQR 44-107 months) of median follow-up. Median TTP was 14 months (IQR 6 – 27 months). In the group of patients who stopped trastuzumab, more patients were diagnosed with oligo-metastases and received LAT for the primary tumor and metastases (not formally tested; Supplementary Table 1).

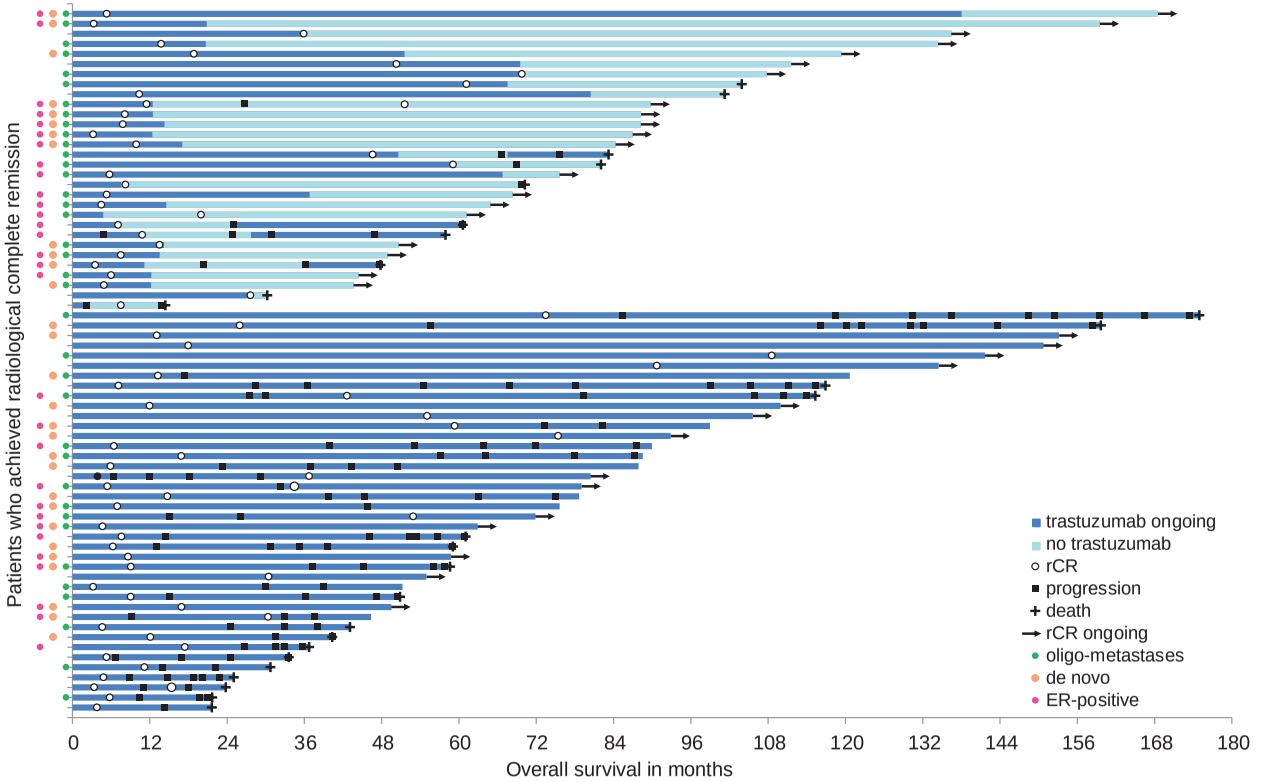

Figure 2. Swimmersplot of all patients who achieved radiological complete remission upon trastuzumab-based treatment (n= 70)

Abbreviations: MBC, metastatic breast cancer; rCR, radiological complete remission; ER, estrogen receptor. Two patients achieved rCR upon hormonal treatment and are not included in this figure.

DISCUSSION

Trastuzumab has dramatically improved survival for patients with HER2-positive MBC with some patients surviving over 10 years. Our multicenter analysis in > 700 patients treated with trastuzumab-based systemic therapy for HER2-positive MBC shows that patients who achieve rCR have a very favorable prognosis compared to patients without rCR. Other factors associated with improved outcome are de novo MBC, longer interval from breast cancer diagnosis until MBC, single-organ metastases, ER-positivity, no liver metastases, no brain metastases, no skin metastases, no prior adjuvant treatment with trastuzumab and successful LAT of metastases. Patients characteristic of our cohort are in line with patients with HER2-positive MBC in other multicenter cohorts.[9, 16, 17] Outcome for patients in our cohort in terms of median OS (37 months) and probability to achieve rCR (10%) is also comparable to other cohorts.[9, 15, 24, 25] With first-line dual HER2-blockade for all patients diagnosed with HER2-positive MBC today, the percentage of patients achieving rCR may increase.

Patients and oncologists are reluctant to stop treatment and generally continue trastuzumab even if rCR is achieved; thereby accepting frequent hospital visits, possible toxicity, and associated drug costs. If the disease is in definitive remission or controlled by immune surveillance, consolidation treatment might no longer be necessary. Whether and when trastuzumab could be discontinued without compromising prognosis is a matter of a clinical debate that is unfortunately not yet supported by randomized-controlled trials data.[9, 18, 26, 27, 32] Among the 70 patients in our cohort who achieved rCR upon trastuzumab-based treatment, 30 patients (43%) discontinued trastuzumab after rCR at the discretion of the treating physician. Most patients discontinued trastuzumab after completing one-year trastuzumab treatment since diagnosis of MBC or ongoing remission upon systemic treatment. Of them, 20 patients (67%) remain disease-free for several years and another two patients died due to another cause than breast cancer while being in remission. In patients who experienced progression after discontinuation of trastuzumab, trastuzumab was restarted in five, resulting in rCR in one patient and stable disease in four patients. Based on our data we cannot draw conclusions whether all patients who achieve rCR should continue trastuzumab or not. To optimally answer this important question, patients should be randomly allocated to either continuation or discontinuation. However, as such a study is unlikely to be performed, a validation of our findings in an independent prospective cohort of patients who have achieved rCR can strengthen our findings.

In our cohort, patients with oligo-MBC (≤ 3 metastases) or de novo MBC were most likely to achieve rCR. Wong *et al.* also found that a few metastatic sites (1-3) were associated with achieving NED in a cohort of HER2-positive de novo MBC.[24] However, the definition

of NED they used is slightly different from what we classified as rCR as patients could also achieve NED after surgery of metastases, which is more often performed in patients with few (oligo-) metastases.

LAT for metastases in patients with few metastases was significantly associated with improved survival in our multivariable analysis. Harano *et al.* also observed improved survival after local treatment of metastases.[33] Patients with skin metastases at MBC diagnosis have a worse OS compared to patients with visceral, bone or CNS metastases. To the best of our knowledge this has not been described before.

There are some limitations that have to be considered when interpreting our results. Firstly, our cohort involves historic data and data collection was not prospectively planned, which may have caused incomplete data or information bias. Secondly, HER2-status was based on assessment at time of initial breast cancer diagnosis, which does not necessarily correspond to HER2 status of the metastatic lesions. Ten percent discordance for HER2-status between primary tumor and metachronous metastases has been reported.[34] Thirdly, analysis regarding LAT for metastases and rCR can be subject to selection bias and immortal-time bias.[35] Most patients who received local treatment of metastases are selected based on remarkable or durable response. Treatment with a multimodality approach, including LAT for metastases, for patients with oligo-MBC was used in some but not all institutes; this variation might have influenced results. Additionally, patients who underwent LAT of metastases did clearly not die before they underwent local treatment, resulting in a biased longer survival time for this group. Fourthly, part of the patients in our cohort (n = 405, 57%) diagnosed with metachronous MBC did not previously receive neoadjuvant or adjuvant trastuzumab, while currently almost all patients who develop metachronous metastases receive neoadjuvant or adjuvant trastuzumab. In our analysis and in previous studies, response to treatment and outcome is different in patients who have received prior trastuzumab.[21] As the majority of patients with metachronous MBC did receive trastuzumab in the neoadjuvant or adjuvant setting, we think our data are important for today's HER2-positive MBC patients. Lastly, only 22 patients (3%) in our cohort received dual HER2-targeted therapy, which is the current standard of care for patients with HER2-positive MBC. Despite these limitations, with a unique long follow-up of this multicenter cohort, we were able to confirm clinical characteristics associated with improved survival.

Conclusion

This large multicenter cohort of 717 patients with HER2-positive MBC shows that achieving radiologic complete remission has important prognostic value. The 10-year OS estimate for patients who achieved rCR was 52% versus 7% for patients who did not

achieve rCR. These findings support a treatment strategy that increases the likelihood of achieving rCR and thus optimizing the chance for long-term survival. However, strong treatment recommendation cannot be made in view of the observational nature of the data. Decisions to discontinue trastuzumab in case of rCR should be carefully considered between physician and patient until more research has been performed.

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SUPPLEMENTAL MATERIAL
CHAPTER 4

Table S1. Baseline characteristics for patients who achieved rCR upon treatment with trastuzumab (n=70)

Baseline characteristics	Patients who discontinued trastuzumab (n=30)	Patients who continued trastuzumab (n=40)	P-value[a]
Age at diagnosis MBC, years			0.628
Median (IQR)	59 (46-62)	48 (42-60)	
Time until MBC, months			0.142
Median (IQR)	21 (0-83)	15 (0-52)	
De novo MBC, no. (%)			1.00
De novo MBC	13 (40)	17 (42)	
Metachronous MBC	18 (60)	23 (58)	
ER-status at diagnosis, no. (%)			0.054
ER-positive	17 (57)	13 (33)	
ER-negative	13 (43)	27 (67)	
Single organ metastases, no. (%)			0.187
Single organ metastases	6 (20)	15 (37)	
Multiple organ metastases	24 (80)	25 (63)	
Oligo-metastases (≤ 3 metastases), no. (%)			0.016
Oligo-metastases	21 (70)	16 (40)	
Multiple metastases	9 (30)	24 (60)	
Location of metastases at diagnosis MBC, no. (%)			
Bone	8 (27)	18 (45)	0.139
Liver	5 (17)	13 (33)	0.172
Lymph nodes	14 (47)	17 (43)	0.810
Lung	4 (13)	10 (26)	0.366
Skin	2 (7)	3 (8)	0.893
CNS	1 (3)	0 (0)	0.245
Prior trastuzumab, no. (%)			0.107
Yes	1 (3)	6 (15)	
No	29 (97)	34 (85)	
Moment first trastuzumab for MBC, no. (%)			1.00
Trastuzumab received in 1st line	26 (90)	36 (90)	
Trastuzumab received in 2nd line	3 (10)	4 (10)	

Table S1. Continued

Baseline characteristics	Patients who discontinued trastuzumab (n=30)	Patients who continued trastuzumab (n=40)	P-value[a]
Successful local ablative treatment of metastases, no. (%)			< 0.001
Yes	17 (57)	6 (15)	
No	13 (43)	34 (85)	

Abbreviations MBC, metastatic breast cancer; IQR, interquartile range; ER, estrogen receptor; CNS, central nervous system.
Two patients achieved rCR upon hormonal treatment and are not included in this table.
Percentages are based on known values.
[a] P-values are based on T-test, Chi-square test of Fisher's exact test, whichever was appropriate for the data.

Table S2. Hazard ratios for death – univariable Cox regression analyses

	HR	95% CI	P-value
Age at diagnosis MBC (years)	1.01	0.99-1.01	0.122
Time until MBC (months)	0.99	0.99-1.00	0.012
ER-positive	0.78	0.66-0.92	0.003
Oligo-metastases (≤ 3 metastases)	0.51	0.41-0.64	< 0.001
Single organ metastases	0.59	0.50-0.70	< 0.001
Bone metastases	1.12	0.95-1.32	0.194
Liver metastases	1.56	1.32-1.84	< 0.001
Lung metastases	1.19	0.99-1.41	0.053
Lymph node metastases	0.97	0.82-1.15	0.751
Skin metastases	1.62	1.20-2.17	0.001
CNS metastases	1.59	1.10-2.28	0.013
Prior trastuzumab			
Prior trastuzumab	1	NA	NA
No adjuvant trastuzumab	0.64	0.51-0.80	< 0.001
No prior trastuzumab - de novo MBC	0.52	0.40-0.67	< 0.001
Radiologic complete remission	0.25	0.17-0.37	< 0.001
Successful local ablative treatment of metastases	0.34	0.23-0.49	< 0.001
Trastuzumab second line	0.89	0.75-1.07	0.209

Abbreviations: HR, hazard ratio; CI, confidence interval; MBC, metastatic breast cancer; IQR, interquartile range; ER, estrogen receptor; CNS, central nervous system; NA, not applicable.

Table S3. Odds ratios for achieving radiological complete remission – univariable logistic regression analyses.

	OR	95% CI	P-value
Age at diagnosis MBC (years)	0.99	0.97-1.01	0.460
Time until MBC (months)	0.99	0.99-1.00	0.741
ER-positive	0.60	0.37-0.98	0.042
Oligo-metastases (≤ 3 metastases)	5.20	3.13-8.63	< 0.001
Single organ metastases	2.78	1.65-4.67	< 0.001
Bone metastases	0.42	0.26-0.70	0.001
Liver metastases	0.49	0.28-0.85	0.011
Lymph node metastases	1.46	0.89-2.39	0.132
Lung metastases	0.52	0.29-0.93	0.028
Skin metastases	1.00	0.38-2.59	0.992
CNS metastases	0.25	0.03-1.88	0.179
Prior trastuzumab			
Prior trastuzumab	1	NA	NA
No adjuvant trastuzumab	1.56	0.65-3.60	0.297
No prior trastuzumab - de novo MBC	2.88	1.22-6.81	0.016
Trastuzumab second line	0.34	0.16-0.69	0.003

Abbreviations: OR, odds ratio; CI, confidence interval; MBC, metastatic breast cancer; ER, estrogen receptor; NA, not applicable.

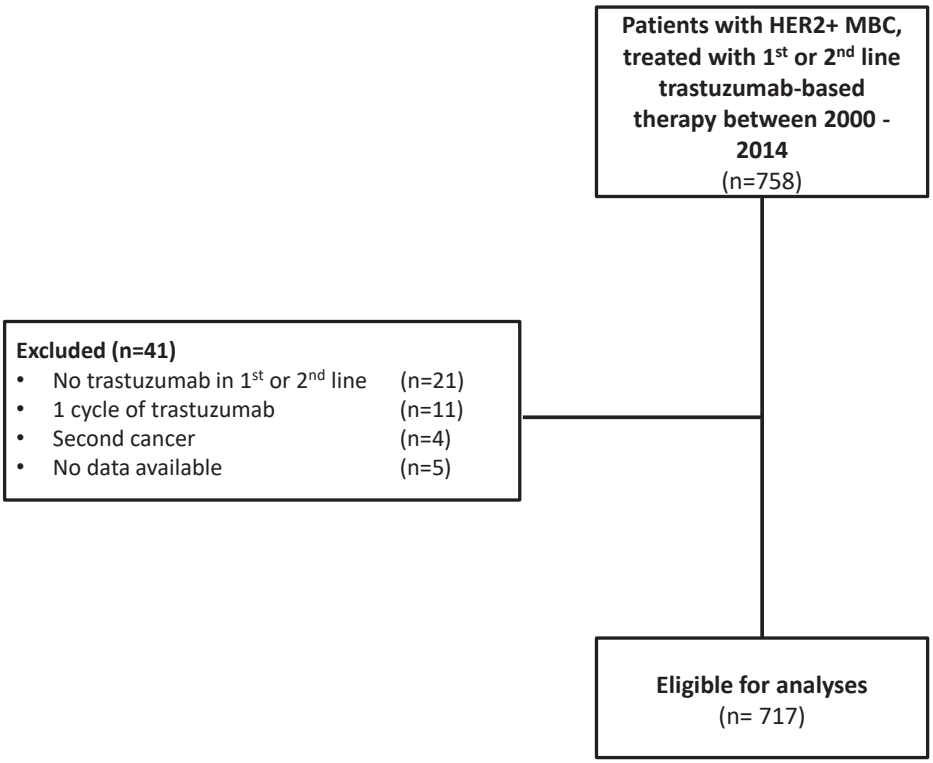
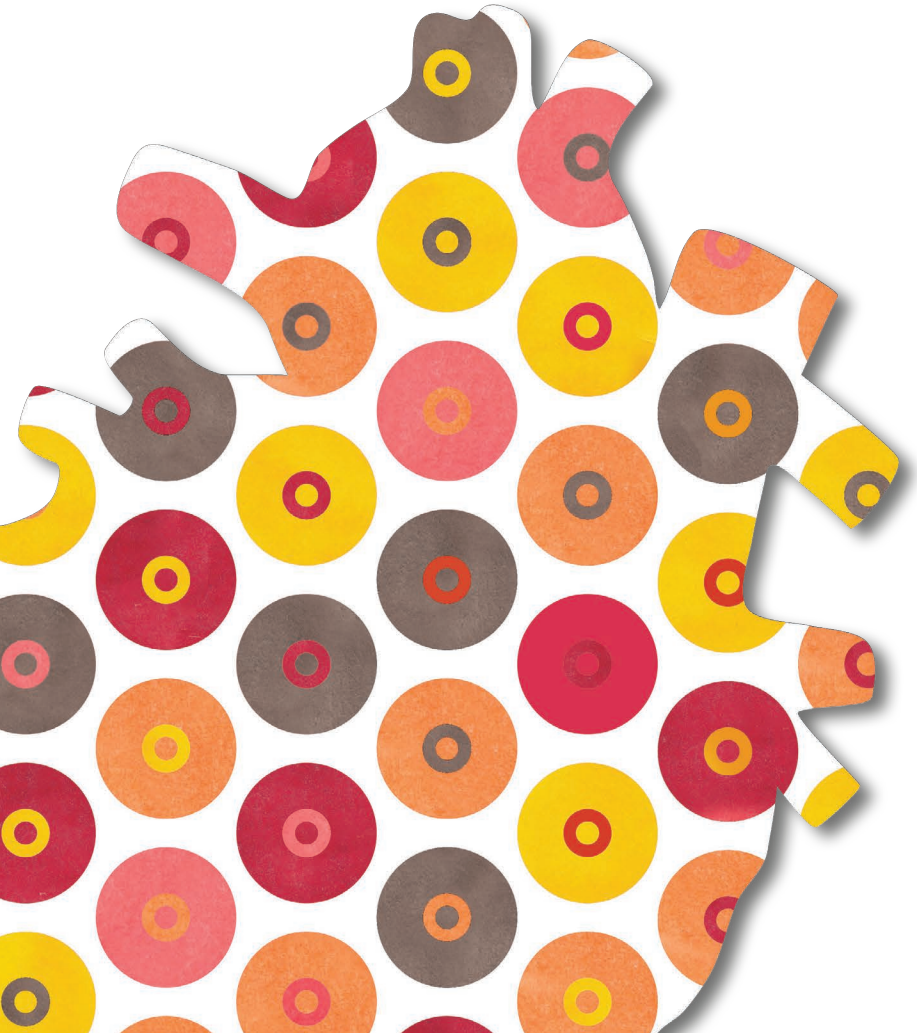


Figure S1. Consort diagram patients eligible for analyses

Abbreviations: HER2, human epidermal growth factor receptor 2; MBC, metastatic breast cancer.

PART II

CARDIAC MONITORING OF PATIENTS WITH HER2-POSITIVE BREAST CANCER DURING TRASTUZUMAB TREATMENT



CHAPTER 5

Cardiac monitoring in HER2-positive patients on trastuzumab treatment: a review and implications for clinical practice

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ABSTRACT

Trastuzumab prolongs progression-free survival and overall survival in patients with human epidermal growth factor receptor-2 (HER2) positive breast cancer. However, trastuzumab treatment is hampered by cardiotoxicity, defined as a left ventricular ejection fraction (LVEF) decline with a reported incidence ranging from 3 to 27% depending on variable factors. Early identification of patients at increased risk of trastuzumab-induced myocardial damage is of great importance to prevent deterioration to irreversible cardiotoxicity. Although current cardiac monitoring with multi gated acquisition scan (MUGA) and/or conventional 2D-echocardiography (2DE) have a high availability, their reproducibility are modest, and more sensitive and reliable techniques are needed such as 3D-echocardiography (3DE) and speckle tracking echocardiography (STE). But which other diagnostic imaging modalities are available for patients before and during trastuzumab treatment? In addition, what is the optimal frequency and duration of cardiac monitoring? At last, which biomarker monitoring strategies are currently available for the identification of cardiotoxicity in patients treated with trastuzumab?

INTRODUCTION

About 20% [1] of breast tumors show overexpression of human epidermal growth factor receptor-2 (HER2), which is caused by amplification of the HER2-oncogene.[2] HER2 is a transmembrane receptor with an intra- and extracellular domain which plays an important role in normal growth and in the development of various tissues.[3, 4] In HER2-overexpressing breast tumors, HER2 is often the main driver through which rapid growth occurs resulting in a poor prognosis.[5, 6]

Trastuzumab is a humanized monoclonal antibody targeting the extracellular domain of HER2. After binding to the extracellular domain of HER2, trastuzumab inhibits the intracellular tyrosine kinase activity and thereby inhibiting the proliferation of HER2-positive breast cancers resulting in cell death. Therefore, trastuzumab is highly effective in patients with HER2-overexpressing breast cancer. Large randomized phase 3 trials showed that addition of one year of trastuzumab treatment to adjuvant chemotherapy impressively improves overall survival by 24 to 33% in these patients.[7, 8] Likewise, addition of trastuzumab to chemotherapy in first line setting for advanced breast cancer increases the overall survival by 5 to 8 months [8, 9] and even up to 15 months when combined with pertuzumab and docetaxel in first line relapse setting.[10]

HER2 is also physiologically expressed on myocytes. Although HER2 is not overexpressed on myocytes, trastuzumab treatment is associated with an increased risk of a decrease in left ventricular ejection fraction (LVEF) which can lead to clinically manifest heart failure. Risk factors for trastuzumab-induced cardiotoxicity are older age (>65 years), hypertension, diabetes mellitus, obesity (BMI >30 kg/m^2), previous anthracycline exposure, short time between anthracycline treatment and anti-HER2 treatment, previous radiation therapy and compromised cardiac function before treatment.[11-15] Because of the impressive prognostic impact of trastuzumab treatment, a sizeable number of patients who survive HER2-positive breast cancer due to trastuzumab treatment are at risk for developing cardiotoxicity. Hence, strategies to monitor and prevent long-term disabling cardiotoxicity are of utmost importance.

Although trastuzumab-induced cardiotoxicity is believed to be reversible, some reports suggest that in about 50% it is only partly reversible and in 12 to 29% it is even irreversible .[16-19] It should be mentioned that these patients included in these reports were all pre-treated with anthracycline, which is a known cause of irreversible cardiotoxicity.[20] Early detection of trastuzumab-derived cardiotoxicity might prevent both reversible and possibly irreversible effects on the heart function [21], because early discontinuation of trastuzumab and/or early implementation of cardio-protective therapies positively

impact cardiac outcome.[22, 23] Importantly, most patients successfully restart trastuzumab treatment after transient LVEF impairment as it has been shown that trastuzumab does not induce permanent myocyte apoptosis as opposed to anthracycline that induce cardiomyocyte apoptosis via oxidative stress and free radical formation.[24, 25] However, recent studies in human cardiac cell cultures and in mice indicate that trastuzumab can induce myocyte apoptosis leading to irreversible cardiotoxicity.[26-28]

The exact mechanism of trastuzumab-induced cardiotoxicity is still unknown. Some studies show that trastuzumab inhibits cardiomyocyte repair by blocking neuregulin-1 and the HER2 downstream pathway which is required for cardiac repair, especially after anthracycline treatment.[29, 30] Another study showed that trastuzumab inhibits topoisomerase IIB, similar to anthracycline, leading to increased reactive oxygen species formation and sequential apoptosis.[31] More research is needed to clearly understand the mechanism of trastuzumab-induced cardiotoxicity.

Thus, LVEF monitoring during trastuzumab is important. However, current cardiac monitoring techniques have some important limitations. First, LVEF measurements vary between the different techniques used in clinical practice and many techniques have questionable reproducibility. Second, LVEF reflects the functional status of the left ventricle (LV) and an LVEF decline is only observed once functional impairment already has occurred. Third, the LVEF is preload and afterload dependent and can substantially change based on different loading conditions.[32] Therefore, for the early detection of injured myocardial cells more sensitive diagnostic tools are required.

In this review, we present an overview and critical appraisal of the state-of-the-art with respect to the role, frequency and duration of cardiac imaging and biomarker monitoring strategies in trastuzumab-treated HER2-positive breast cancer patients.

What is the incidence of cardiotoxicity of trastuzumab?

The incidence of cardiotoxicity due to trastuzumab treatment varies according to the definition used, whether or not trastuzumab is combined with anthracycline (sequential or concomitant) and/or other HER2-blocking agents such as pertuzumab and lapatinib which have similar to lower risk of cardiotoxicity (Table 1), treatment duration, and type of imaging technique used. Most trials use the definition of cardiotoxicity related to cancer therapeutics defined by the European Society of Cardiology (ESC) as a decrease in LVEF of >10%-points to a value below 50%.[33] However, consensus on the definition of cardiotoxicity is missing. In this review, the term cardiotoxicity refers to myocardial damage related to anticancer pharmacological therapies.

Trastuzumab monotherapy in adjuvant setting has a relatively low incidence of trastuzumab-related cardiotoxicity of 3 to 7%, however when given concomitantly with doxorubicin and cyclophosphamide the reported incidence of cardiotoxicity can increase up to 27% in clinical trials.[34] This seems due to cumulative toxicity of simultaneous administration of these agents as the repair of oxidative stress induced by anthracycline is hampered by trastuzumab blocking the HER2 downstream pathway that is required for cardiac repair.[35] Interestingly, concomitant use of trastuzumab and epirubicin appears to be far less cardiotoxic [36, 37], resulting in an increased use even in the neo-adjuvant setting. Although both agents are anthracycline, it remains unknown why epirubicin, a 4'-epimer of doxorubicin, is less cardiotoxic.[38]

An important issue to address is whether these incidence rates are similar to those among older patients (>70 years), patients with comorbidities and with a history of cardiac disease or heart failure who are excluded in most clinical trials. Population based, retrospective studies indicate that the rates and impact of trastuzumab-induced cardiotoxicity might be higher in 'real world' patients than mentioned in clinical trials.[39] For example, the 5-year cumulative incidence of cardiotoxicity in older patients (>75 years) treated with anthracycline and trastuzumab was 41% compared to incidence of 27% found in a clinical trial.[34, 40] Therefore, the incidence rates of these selected patients from prospective clinical trials cannot be unconditionally extrapolated to 'real world' patients.

Which diagnostic imaging modalities are currently available for patients on trastuzumab treatment?

MUGA scan

Multigated acquisition (MUGA) scan is a frequently used imaging modality for evaluation of LVEF evolvement during chemotherapy and trastuzumab therapy. MUGA scan is a minimal-invasive nuclear imaging technique using ^{99m}Tc-erythrocyte labeling. It is capable to measure systolic-diastolic changes in radioactivity within the LV to calculate the blood flow leaving the LV, i.e. the LVEF. This method has a high availability and few technical limitations as it can be performed in all patients without limitations due to obesity, poor acoustic windows or presence of cardiac devices such as pacemakers or defibrillators (see Table 2).[45] Nevertheless, major limitations of measuring the LVEF with MUGA scans are the questionable accuracy, modest reproducibility, cumulative radiation exposure of serial monitoring, and its limited information on cardiac structural dysfunction.[46] The accuracy of MUGA scans remains dubious as contemporary MUGA scans are performed with a large-field-of-view, two head system gamma cameras that do not allow optimal patient positioning.[47] In addition, assessment of the LVEF with MUGA scans has shown to

have large inter-and intra-observer variations that varies between hospital centers and software packages computer processing systems, where in the latter the variation in LVEF can be 5.1%-points.[48, 49]

Besides this, the radiation exposure from a single MUGA scan is approximately 10mSv per scan, which is three times the average yearly background radiation.[46] Thus, serial monitoring of the LVEF results in an unacceptable high cumulative exposure, while most breast cancer patients already have an increased radiation exposure due to other imaging modalities used for staging of the breast cancer. Fortunately, new 3D MUGA cameras allow obtaining the LVEF with less than 2mSv [50], but these cameras are not yet implemented in daily clinical practice.

Echocardiography, ultrasound of the heart
Cardiac evaluation before, during and after trastuzumab treatment can also be performed with conventional echocardiography. Advantages of echocardiography over MUGA are its lack of radiation exposure and the possibility to assess the complete cardiac structure. Disadvantages of echocardiography are the high inter-observer variability, dependency on image quality, insensitivity to detect small LVEF changes and a false positive rate of cardiotoxicity of 3.6%.[51]

Three-dimensional echocardiography (3DE) has a better accuracy in detecting LVEF below the lower limit of normal and a better reproducibility than the conventional two-dimensional echocardiography (2DE) biplane Simpson method.[52, 53] However, applicability of 3DE in the oncologic setting is limited, because it remains dependent on availability in the different hospitals, requires adequate image quality (more so than 2DE) and operator experience (Table 2).

Speckle tracking echocardiography
A sensitive and promising diagnostic modality is the so-called strain imaging with speckle tracking echocardiography (STE).[47] Dedicated STE software is capable to track the movement of speckles, which are tracking points that are placed automatically on the LV wall in the conventional 2D-echocardiographic images and reflect the movement of the different segments of the heart.[54] Additionally, STE can also be applied in 3DE and CMR, which is currently not frequently observed in clinical practice.[55] Strain is a method for measuring regional or global myocardial deformation, i.e. the proportional change in dimension of the heart in relation to the original dimension of the heart. Consequently, strain measurement with STE is capable to quantify myocardial function regionally, and to identify subtle and subclinical LV dysfunction. New parameters for myocardial function assessment emerged with the introducing of STE, including global longitudinal

strain (GLS), global circumferential strain (GCS) and global radial strain (GRS). The GLS reflects the longitudinal contraction of the myocardium, and thus represents the global left ventricular function.[56, 57] The GCS reflects the circumferential contraction of the myocardium and is expressed as a negative percentage value, likewise as the GLS, as the myocardium shortens during contractions. Finally, the GRS reflects the radial contraction of the myocardium, i.e. the lengthening of the myocardium and is expressed as a positive percentage. In the general population, GLS has a better prognostic value than 2DE-LVEF to predict major adverse cardiac events including hospitalisation due to heart failure and cardiac death during an average follow-up of 27 months.[58]

Table 1. Incidence of cardiotoxicity varying per chemotherapeutic and definition used

Exposed chemotherapeutic	Incidence	Cardiotoxicity definition used:
Trastuzumab monotherapy [34]	3-7%	CREC criteria: 1. Cardiomyopathy characterized by a decrease in cardiac LVEF global or severe in the septum 2. Symptoms of CHF 3. Associated sign of CHF, including S3 gallop, tachycardia or both 4. Symptomatic decline in LVEF >5% to an LVEF <55% or an asymptomatic LVEF decline of >10% to an LVEF <55%.
Trastuzumab with previous anthracycline: epirubicin [41]	4%	CHF
Trastuzumab with previous anthracycline: doxorubicin [34]	13%	CREC criteria as mentioned above.
Trastuzumab, epirubicin and cyclophosphamide [42]	5%	Symptomatic heart failure NYHA class III or IV associated with an absolute decrease in LVEF of more than 10% points to less than 50%
Trastuzumab, doxorubicin and cyclophosphamide [34]	27%	CREC criteria as mentioned above.
Trastuzumab + HER2 inhibitor: pertuzumab [43]	4%	LVEF of less than 50% and a decrease of more than 10% from baseline
Trastuzumab + intracellular HER2-kinase inhibitor: lapatinib [44]	3%	LVEF of less than 50% and a decrease of more than 10% from baseline or congestive heart failure or myocardial ischemia

Abbreviations: CREC, Cardiac Review and Evaluation Committee; LVEF, left ventricular ejection fraction; CHF, congestive heart failure; NYHA, New York Heart Association; HER, human epidermal growth factor receptor.

Table 2. Various imaging techniques for the detection of cardiotoxicity

Imaging technique	Advantages	Limitations
Echocardiography • Two-dimensional • Three-dimensional	Wide availability Lack of radiation exposure Cost-effective Assessment of myocardial structures High accuracy in detecting small LVEF changes High reproducibility compared to 2DE Lack of radiation exposure Cost-effective Assessment of myocardial structures	High inter-observer variability Dependent on image quality Insensitive to detect small LVEF changes Dependent on image quality and operator experience Low availability in cancer centers
Speckle tracking echocardiography	GLS for subclinical identification of cardiotoxicity Relatively low angle dependence Same advantages as echocardiography	Inter-vendor variability Technical requirements Dependent on image quality Low temporal resolution Different results due to different algorithms
Cardiac magnetic resonance	High accuracy High reproducibility Assessment of myocardial structures/ myocardial fibrosis Helps identifying the cause of cardiotoxicity Low radiation exposure	Limited availability High costs Low temporal resolution Patients' adaptation (claustrophobia, breath hold and long acquisition times) Limited in patients with metallic prosthetics
MUGA scan	Availability Few technical limitations	High cumulative radiation exposure Limited informative on cardiac structures Lower accuracy

Abbreviations: LVEF, left ventricular ejection fraction; MUGA, multi-gated acquisition scan.

Which new diagnostic imaging modalities are available for patients on trastuzumab treatment?

In patients with HER2-positive breast cancer treated with anthracyclines and trastuzumab an GLS decline from baseline measured with STE precedes an LVEF decline measured with 2DE.[59-62] The systematic review of Thavendiranathan *et al.* demonstrated that an early relative reduction in GLS of ≥10% during therapy is very likely to be indicative for cardiotoxicity defined as an LVEF decline of ≥5% to an LVEF <55% or symptomatic heart failure.[63] In addition, the expert consensus document of the American Society of Echocardiography (ASE) and European Association for Cardiovascular Imaging (EACVI) suggests that a relative change in GLS >15% from baseline is likely to indicate of clinically meaningful cardiotoxicity.[47] Likewise, a recent meta-analysis showed that the absolute GLS cut-off values, ranging from -21 to -14%, can be useful to stratify patients at high risk of developing cardiotoxicity which is important in patients without baseline cardiac imaging.[64] Although a reduction in GLS is correlated with an reduction in LVEF [59-62], little is known about the predictive value of an impaired GLS on long term cardiotoxicity, the optimal GLS cut-off value and the development of an impaired GLS to symptomatic heart failure. Importantly, STE has some disadvantages including the need for high-resolution image quality and the insufficient standardization of measurements by different echocardiographic vendors resulting in non-comparative results.[33]

Thus, the role of speckle tracking echocardiography in early detection of subclinical cardiotoxicity in the oncologic setting seems promising. However, these findings should be validated in larger prospective multicenter studies. The results of the SUCCOUR trial, which is a currently ongoing randomized controlled trial that randomly allocates patients on cardiotoxic cancer treatment to an GLS-guided treatment strategy or LVEF-guided treatment strategy, are urgently awaited in this respect.[65]

Cardiac MRI

Cardiac magnetic resonance (CMR) imaging is considered the gold standard for evaluation of the cardiac structure and cardiac function. Besides quantification of cardiac dysfunction, CMR provides insight in its likely cause, as it provides information about the cardiac structure and myocardial fibrosis because of its high resolution.[66] Other advantages of CMR are its high reproducibility and accuracy. The major limitations of CMR however, are its high costs and therefore limited availability, the reason why CMR is currently not used for serial monitoring of the LVEF.

Comparison of imaging modalities and summary

LVEF assessments with MUGA scan, echocardiography and CMR show poor correlation.[67] Discordance between the MUGA and CMR in measuring the LVEF particularly occurred in small left ventricles, small hearts and heart with a history of atrial fibrillation.[68] Furthermore, 2DE is suggested to overestimate the mean LVEF with 5% and has a lower sensitivity in detecting LVEF values less than 50% compared to CMR.[69] Possible false-positive results due to measuring the LVEF with echocardiography or MUGA cannot be excluded. In contrast to conventional 2DE, 2DE-ST is highly correlated with CMR in measuring the LVEF.[70] Comparisons of 2D-STE with 3DE-ST showed that 3DE-ST had lower inter- and intra-observer variability.[71] Therefore, it is recommended to use the same monitoring modality for baseline and follow-up LVEF assessments.[33, 72]

Concluding, not 2DE or MUGA scan but 3DE seems most suitable for cardiac monitoring of patients on trastuzumab due to the high reproducibility, high accuracy and the ability to assess the complete cardiac structure. In addition, strain measurement with STE seems promising to detect myocardial damage in an early stage compared to LVEF measurement.

What is the optimal frequency and duration of cardiac monitoring for patients on trastuzumab?

Another important question for clinicians who treat patients on trastuzumab is: what is the optimal frequency and duration of cardiac monitoring? The US Food and Drug Administration (FDA) label of trastuzumab recommends, based on protocols in clinical trials, LVEF monitoring prior to initiation of trastuzumab and every 3 months during and upon completion of adjuvant trastuzumab treatment. After completion of 1 year trastuzumab in the adjuvant setting, LVEF monitoring every 6 months for at least 2 years is recommended. If trastuzumab is withheld for significant left ventricular cardiac dysfunction, cardiac monitoring should occur monthly. These recommendations have been adopted into clinical practical guidelines by the European Society of Medical Oncology (ESMO) [73], European Society of Cardiology (ESC) [33] and American Society of Clinical Oncology (ASCO).[11] The ESC differentiates between low risk patients (normal baseline echocardiogram and no clinical risk factors) for whom cardiac monitoring every 3 months is advised and high risk patients (abnormal baseline echocardiography and clinical risk factors) for whom even more frequent cardiac monitoring is advised. Interestingly, it should be noted that the ASCO recommends cardiac monitoring at 6 to 12 months after completion of therapy in patients at high risk. Lastly, the ESMO recommends cardiac monitoring infrequently in the absence of symptoms during trastuzumab for metastatic breast cancer. As these guidelines are mainly based on expert consensus rather than trial data and as they have not been prospectively validated, the optimal frequency of cardiac monitoring during trastuzumab treatment has not been established yet.

Table 3 gives an overview of frequency and duration of cardiac monitoring during and after trastuzumab treatment from current literature. In cohort studies, cardiac monitoring was most often performed every 3 to 4 months in the first year of trastuzumab treatment for early-stage breast cancer. However, after discontinuation of trastuzumab for early-stage breast cancer, cardiac monitoring was only observed in a few studies (4 out of the 12 studies). During trastuzumab treatment for advanced-stage disease, cardiac monitoring was performed every 3 to 8 months up until 2 years of trastuzumab treatment. After 2 years of trastuzumab treatment, cardiac monitoring was only observed in 1 study.[74] In addition, in randomized controlled trials, cardiac monitoring was performed every 3 to 6 months during the first year of trastuzumab treatment or even 2 years in some studies.[9, 10, 75, 76] After discontinuation of trastuzumab, cardiac monitoring was performed in the majority of the studies (10 out of the 15 studies), which was not often observed in the cohort studies. Studies investigating cardiac monitoring after discontinuation of trastuzumab showed a low cumulative incidence of cardiotoxicity, defined as LVEF decline >10%-points from baseline to LVEF <50% or <55% or New York Heart Association (NYHA) class III or IV, of 0.5 to 5.8%.[19, 77, 78] However, conflicting results are found regarding the development of heart failure 2 years after starting trastuzumab. An 2-fold increased risk of late heart failure of trastuzumab compared to chemotherapy alone has been found in one study [79], whereas other studies did not find excess risk of late heart failure after trastuzumab discontinuation.[80, 81] Concluding, cardiac monitoring, predominantly with echocardiography or MUGA, occurs most often every 3 months during trastuzumab treatment for early-stage breast cancer, and more infrequently during trastuzumab treatment for advanced-stage breast cancer. Based on current knowledge, the additional value of frequent cardiac monitoring after discontinuation of trastuzumab has not been confirmed. It would be reasonable to consider a less frequent schedule or omission of monitoring after discontinuation of trastuzumab.

Table 3. Overview of studies in which cardiotoxicity was monitored during and after trastuzumab treatment

Study	n	Stage	Treatment	Cardiac monitoring modality	During trastuzumab treatment		
					Baseline	0-3 months	3-6 months
Cohort							
Tarantini *et al.* (2012)[122]	499	Early	AC + Tax + T	Echo	+	+	+
Piotrowski *et al.* (2012)[123]	253	Early	AC + Tax + T	2DE	+	+	+
Cochet *et al.* (2011)[124]	118	Early	AC + Tax + T	Radionuclide angiography	+	+	+
Dent *et al.* (2012)[125]	48	Early	AC + T	Echo and MUGA	+	+	+
Matos *et al.* (2016)[126]	92	Early	AC + T	2DE	+	-	+
Seferina *et al.* (2016)[127]	230	Early	AC + Tax + T	Unknown	+	+	+
Visser *et al.* (2016)[128]	171	Early	Chemo + T	MUGA	+ ↓76%	+ ↓53%	+ ↓40%
Chavez *et al.* (2015)[129]	2203	Early	Chemo + T	Echo or MUGA	+ ↓79%	+ ↓87%	+
Lidbrink *et al.* (2019)[130]	3733	Early	AC + Tax + T	Echo, MUGA, CMR or other	+	+	+
Grazziotin *et al.* (2017)[131]	109	Early/ advanced	AC + Tax + T	Echo	+	+	+
Sun *et al.* (2016)[74]	105	Early/ advanced	AC + T	Echo	+	+	+

	6-12 months	1-2 years	>2 years	Monthly interval	After trastuzumab			Cardiotoxicity incidence	Definition of cardiotoxicity	Additional remarks
					0-3 months	3-6 months	> 6 months			
	+	NA	NA	3	-	-	-	27%	LVEF decline >10% or <50% or heart failure	
	+	NA	NA	3	+	+		21%	LVEF decline >15%, or >10% to <50% or signs of heart failure	
	+	NA	NA	3	-	-	-	15%	Asymptomatic LVEF decline >10%	
	+	NA	NA	3	-	-	-	59%	LVEF decline ≥10 or heart failure	Repeat monitoring as clinically indicated
	+	NA	NA	4	-	-	-	21%	LVEF decline >10% or signs of heart failure	
	+	NA	NA	3	-	-	-	13%	LVEF decline >10% to <50%	
	+ [1]30%	NA	NA	3	-	+ [1]9%	+ [1]1%	NA	NA	
	+	NA	NA	4 [1](43%)	+	+	-	NA	NA	
		NA	NA	3	-	+	+	3%	Symptomatic heart failure	Until 2 years after stop T
	+	-	-	3-4	-	-	-	53%	LVEF decline >10% or <50% or heart failure	Frequency depending on physicians
	+	+	+	3				NA	NA	

Table 3. Continued

Study	n	Stage	Treatment	Cardiac monitoring modality	During trastuzumab treatment		
					Baseline	0-3 months	3-6 months
Davis *et al.* (2016)[132]	43	Early/ advanced	AC + Tax + T	Echo	+ [1]35%	-	-
Extra *et al.* (2010)[133]	623	Advanced	AC + T	Unknown	+ [1]57%	+/-	+/-
RCT							
Perez *et al.* (2008)[78]	2148	Early	AC + P AC + P + T	Echo or MUGA	+	+	+
Suter *et al.* (2007) [134]	3386	Early	AC + T	Echo or MUGA	+	+	+
Romond *et al.* (2005)[7]	3351	Early	AC + P AC + P + T	Echo or MUGA	+	+	+
Joensuu *et al.* (2009)[72]	232	Early	Tax + FEC + T V + FEC + T	(Isotope) echo	+	-	+
Tan-Chiu *et al.* (2005)[19]	2043	Early	AC + P AC + P + T	MUGA scan	+	-	+
Piccart-Gebhart *et al.* (2005) [135]	5081	Early	AC + T	Echo or MUGA	+	+	+
Cameron *et al.* (2017)[77]	5102	Early	AC + Tax + T	Echo or MUGA	+	+	+
Gianni *et al.* (2010)[136]	235	Early	AC + Tax+ T	Echo or MUGA, + ECG	+	-	-
Slamon *et al.* (2011)[137]	3222	Early	ACT-T + T TCH	Echo[2] or MUGA	+	+	-
Swain *et al.* (2015)[10]	808	Advanced	Pe + T + Tax T + Tax	Echo or MUGA s	+	+	+
Blackwell *et al.* (2010)[75]	296	Advanced	AC + Tax + T T + L	Echo or MUGA	+	+	+

6-12 months	1-2 years	>2 years	Monthly interval	After trastuzumab			Cardiotoxicity incidence	Definition of cardiotoxicity	Additional remarks
				0-3 months	3-6 months	> 6 months			
+-	+-	-	8	-	-	-	18%	CREC criteria (Table 1)	
+/-	+	-	No	-	-	-	3%	Heart failure	
+	NA	NA	3	+	+	-	3% 3%	Cardiac event assessed by 3 cardiologists	
+	NA	NA	6	+	+	+	7%	LVEF decline >10% to <50%	
+	NA	NA	NA	-	-	-	4% 3%	NYHA III/IV heart failure after 3 years	
-	NA	NA	NA	-	-	+	1%	Heart failure	Up to 5 years after start T
+	NA	NA	NA	-	+	-	4%	NYHA III/IV heart failure after 3 years	Repeat monitoring as clinically indicated
+	NA	NA	6	-	+	+	1%	Symptomatic heart failure	Up to 5 years after start T
+	NA	NA	6	-	+	+	1%	NYHA III/IV and LVEF decline >10% to <50%	3 years after start T annually to 10 years
-	NA	NA	NA	+	-	-	2%	NYHA III/IV heart failure	
+	NA	NA	NA	-	-	+	19% 11%	Heart failure and LVEF decline >10%	Up to 5 years after start of T
+	+	+	3	+	+	+	6% 7%	LVEF decline >10% to <50%	Up to 1.5 year after stop of T
+	+	+	1	+	-	-	5%	LVEF decline <20%	

Table 3. Continued

Study	n	Stage	Treatment	Cardiac monitoring modality	During trastuzumab treatment		
					Baseline	0-3 months	3-6 months
Marty *et al.* (2005)[9]	186	Advanced	AC + T T + Tax	Echo or MUGA	+	+	+
Von Minckwitz *et al.* (2009)[138]	156	Advanced	T T + C	Echo	+	-	-
Gasparini *et al.* (2007)[139]	123	Advanced	T T + P	Echo or MUGA	+	+	+
Kaufman *et al.* (2009) [76]	207	Advanced	T T + A	Unknown	-	+	+
Müller *et al.* (2018)[140]	19	Advanced	T	Echo or MUGA	-	-	-

Abbreviations: AC, anthracycline + cyclophosphamide; T, trastuzumab; Tax, taxanes; P, paclitaxel; D, doxorubicin; V, vinorelbine; FEC, fluorouracil, epirubicin, docetaxel; Pe, pertuzumab; ACT-T, doxorubicin, cyclophosphamide followed by docetaxel; TCH, doxetaxel, carboplatin and trastuzumab; L, lapatinib; C, capecitabine; A, anastrozole; Echo, echocardiography;

6-12 months	1-2 years	>2 years	Monthly interval	After trastuzumab			Cardiotoxicity incidence	Definition of cardiotoxicity	Additional remarks
				0-3 months	3-6 months	> 6 months			
+	+	+	3	-	-	-	2%	Symptomatic heart failure	
-	-	-	NA	-	-	-	5%	NYHA class III/IV heart failure	Repeat monitoring as clinically indicated
+	-	-	3	-	-	-	0%	Symptomatic heart failure	
+	+	-	2 months	-	-	-	14%	Cardiac events: heart failure, LVEF decline or discontinuation of T	
-	-	-	No				0%	NYHA class II-IV	Monitoring as clinically indicated

MUGA, multi-gated acquisition scan; CMR, cardiac magnetic resonance imaging; NA, not applicable; LVEF, left ventricular ejection fraction; RCT, randomized controlled trial; CREC, Cardiac Review and Evaluation Committee; NYHA, New York Heart Association.

[1]Adherence rate of cardiac monitoring [2] Echocardiography is preferred cardiac monitoring method, same method is advised. + cardiac monitoring performed, - monitoring not performed.

What is the role of cardiac biomarkers in detecting cardiotoxicity?

Troponin

Cardiac troponin regulates the contractile element actin and myosin. It is a protein with three subunits: T, I and C. Troponin I and troponin T are exclusively present in myocardial cells, whereas troponin C is also present in slow-twitch skeletal muscles.[82] Elevated cardiac troponin is indicative of myocardial damage due to for instance acute coronary syndrome or acute myocarditis.[83, 84] Therefore, it can be expected that myocardial damage due to trastuzumab treatment can lead to increased troponin levels.[85]

Studies measuring conventional troponin showed conflicting results (Table 4). Some studies, in which patients received anthracycline and trastuzumab, demonstrate a relationship between increased troponin values during trastuzumab treatment and an LVEF decline.[86, 87] In particular, patients with increased troponin values after anthracycline treatment had higher risk of developing cardiotoxicity than those with stable (low) values.[88] However, most studies did not demonstrate this relationship in patients with trastuzumab-based chemotherapy.[89-94] One of these studies concluded that elevation of troponin preceded changes in LVEF, but not particularly predicted clinical cardiac dysfunction.[93] The most likely explanation for the conflicting results is the timing of conventional troponin measurement [95] and the preceding treatment with anthracycline.[88, 96, 97]

However, the studies that used high-sensitivity (hs) troponin assays demonstrated a relationship between increased hs-troponin values and LVEF.[59, 60, 97, 98] It is plausible that hs-troponin assays have a better range for detection of cardiac dysfunction in patients treated with trastuzumab than conventional troponin assays. Lastly, sex-specific cut-off values of troponin assays have to be taken into account. [99]

NT-proBNP

The N-terminal fragment of the pro-hormone brain natriuretic peptide (BNP), NT-proBNP, is the inactive N-terminal fragment of the biologically active hormone BNP and is secreted by myocytes in response to increased cardiac transmural pressure. NT-proBNP has shown to be useful as a diagnostic and prognostic indicator of heart failure in multiple clinical settings, as summarized in the systematic review by Santaguida *et al.*[103]

Increased NT-proBNP values have been associated with cardiotoxicity in patients treated with anthracycline .[100, 104, 105] Interestingly, another study demonstrated that in patients treated with anthracycline and trastuzumab the risk of LVEF decline increased by almost 30% for each 10 ng/dL increase in NT-proBNP during trastuzumab treatment.[98] However, elevated NT-proBNP values are also seen in patients receiving trastuzumab

with a normal LVEF.[106] Furthermore, not every study demonstrated an association between NT-proBNP and cardiotoxicity during anthracycline and trastuzumab (Table 4).[59, 60, 87, 92, 94, 101] This is possibly due to the wide biological variation (analytical and intra-individual) of natriuretic peptides levels due to secretory burst and rapid turnover.[107]

CRP

C-reactive protein (CRP) is a marker of inflammation which plays a role in atherosclerosis and acute coronary syndrome.[108, 109] It has also been demonstrated to be of prognostic value in patients with chronic heart failure.[110] Therefore, it could be hypothesized that inflammation could also have a role in trastuzumab-induced cardiotoxicity.

A small study of 54 breast cancer patients undergoing trastuzumab therapy revealed that normal hs-CRP values may be associated with low risk of developing a LVEF decline, thus suggesting that hs-CRP might have a high negative predictive value. (Table 4) However, multiple other studies could not demonstrate the relationship.[89, 92, 93]

MPO

Myeloperoxidase (MPO) is a pro-inflammatory enzyme secreted by polymorph nuclear neutrophils that is involved in lipid peroxidation and released in periods of oxidative stress.[111, 112] MPO is associated with increased risk of cardiac problems such as coronary artery disease and heart failure exacerbations.[113, 114] The potential pathophysiological mechanisms of cardiotoxicity due to anthracycline or trastuzumab involves oxidative stress.[115] Therefore, it is biologically plausible that elevated MPO after anthracycline and trastuzumab therapy can be associated with cardiotoxicity.

The study of Ky *et al.* demonstrated that 1 standard deviation increase in MPO during anthracycline treatment in patients with early-staged breast cancer was associated with a 34% increased risk of subsequent cardiotoxicity.[87] In addition, another study found that increases in MPO were associated with cardiotoxicity during anthracycline and trastuzumab treatment.[92]

IgE

Immunoglobulin E (IgE) is involved in the immune defense against parasitic disease and in the pathogenesis of allergic diseases. IgE is synthesized by plasma cells and the expression is controlled by two CD4+ T-helper cells: Th1 and Th2 which have counter-regulatory effects. It is known that the immune system is involved in maintaining myocardial homeostasis in patients with heart failure as it influences myocyte hypertrophy and myocyte loss through apoptosis.[116] The precise role of the immune system in trastuzumab-induced cardiotoxicity is yet unknown.

Table 4. Cardiac) biomarkers for identification of cardiotoxicity

Type cardiac biomarker	Study	Number of patients	Chemotherapy
Troponin T	Fallah-rad *et al.* (2011) [89]	42	AC + T
	Ponde *et al.* (2018) [90]	280	T + L
	Goel *et al.* (2019) [94]	217	AC + T
Troponin I	Cardinale *et al.* (2004) [88]	703	AC + Tax + C
	Cardinale *et al.* (2002) [96]	211	High dose chemotherapy
	Onitilo *et al.* (2012) [91]	54	T
	Cardinale *et al.* (2010) [86]	251	AC + T
	Ky *et al.* (2014) [87]	78	AC + T
	Putt *et al.* (2015) [92]	78	AC + Tax + T
	Morris *et al.* (2011) [93]	59	AC + L + T
Hs-Troponin T	Kitayama *et al.* (2017) [97]	40	AC + T
	Zardavas (2017) [98]	452	AC + T
Hs-Troponin I	Sawaya (2011) [60]	43	AC + T

Time point(s) indicative of cardiotoxicity	Detection of cardiotoxicity?	Definition cardiotoxicity:
12 months after initiation of T treatment	-	Absolute LVEF decline > 10% from baseline to <55% with symptoms of heart failure
Baseline 2 and 18 weeks after initiation of T treatment	-	Symptomatic heart failure NYHA class III or IV, or cardiac death and secondary cardiac events were asymptomatic or symptomatic absolute LVEF decline <50% and >10 points
Baseline, after AC and every 3 months during T	-	Absolute LVEF decline >15% from baseline, or absolute LVEF decline >10% to 50%.
Soon after chemotherapy and 1 month after chemotherapy	+	Death with cardiac cause Acute pulmonary edema Overt heart failure Asymptomatic LVEF reduction ≥ 25% Arrhythmias and conduction disturbances requiring a pacemaker
After high dose chemotherapy	+	Absolute LVEF decline
Baseline vs. every 3 weeks for 1 year T treatment	-	Absolute LVEF decline ≥15% from baseline or an LVEF<50%
Baseline vs. during T treatment	+	Absolute LVEF decline >10% from baseline to <50%
Baseline vs. 3 months after initiation of AC	+	CREC definition of cardiotoxicity
Baseline vs. every 3 months to 15 months after start treatment	-	CREC definition of cardiotoxicity
Maximum levels of: Baseline, 2 months after start AC, 3 months after start AC	-	Maximal absolute LVEF decline (max LVEF –min LVEF / min LVEF) and congestive heart failure
Baseline vs. during AC and/or T treatment	+	Absolute LVEF decline >10% from baseline, symptomatic cardiac failure, acute coronary syndrome or arrhythmias
Baseline	+	Absolute LVEF decline of >10% from baseline to <50%
Baseline vs. completion of AC treatment	+	Absolute LVEF decline of ≥5% to <55% with symptoms of heart failure or an asymptomatic absolute LVEF decline ≥10% to <55%

Table 4. Continued

Type cardiac biomarker	Study	Number of patients	Chemotherapy
	Sawaya (2012) [59]	81	AC + Tax + T
	Zardavas (2017) [98]	452	AC + T
NT-proBNP	Romano *et al.* (2011) [100]	71	AC + Tax + T
	Zardavas *et al.* (2017) [98]	452	AC + T
	Ponde *et al.* (2018) [90]	280	L + T
	Ky *et al.* (2014) [87]	78	AC + T
	Sawaya *et al.* (2011) [60]	43	AC + T
	Putt *et al.* (2015) [92]	78	AC + Tax + T
	Sawaya *et al.* (2012) [59]	81	AC + Tax + T
	Fallah-rad *et al.* (2011) [89]	42	AC + T
	Bouwer *et al.* (2019) [101]	135	AC + T
	Goel *et al.* (2019)[94]	217	AC + T
hs-CRP	Putt *et al.* (2015) [92]	78	AC + Tax + T

Time point(s) indicative of cardiotoxicity	Detection of cardiotoxicity?	Definition cardiotoxicity:
At completion of AC treatment	+	Absolute LVEF decline of ≥5% to <55% with symptoms of heart failure or an asymptomatic absolute LVEF decline ≥10% to <55%
Baseline	+	Absolute LVEF decline of >10% from baseline to <50%
Baseline vs. highest value during chemotherapy	+	Absolute LVEF decline ≥20% and/or increase in LV end systolic volume ≥15% from baseline at 3, 6 and 12 months:
Baseline vs. during T treatment	+	Absolute LVEF decline of >10% from baseline to <50%
Baseline 2 and 18 weeks after initiation of T treatment	-	Symptomatic heart failure NYHA class III or IV, or cardiac death and secondary cardiac events were asymptomatic or symptomatic absolute LVEF decline <50% and >10 points
Baseline vs. 3 months after initiation of AC	-	CREC definition of cardiotoxicity
3 months after initiation of AC treatment Baseline vs. 3 months after initiation of AC treatment	-	Absolute LVEF decline of ≥5% to <55% with symptoms of heart failure or an asymptomatic absolute LVEF decline ≥10% to <55%
Baseline vs. every 3 months to 15 months after start T	-	CREC definition of cardiotoxicity
Baseline vs. for 1 year follow-up	-	Absolute LVEF decline of ≥5% to <55% with symptoms of heart failure or an asymptomatic absolute LVEF decline ≥10% to <55%
12 months after initiation of T treatment	-	Absolute LVEF decline >10% to <55% with symptoms of congestive heart failure.
Baseline vs. during Treatment	-	Absolute LVEF decline >10% and/or LVEF <45%
Baseline, after AC and every 3 months during T	-	Absolute LVEF decline >15% from baseline, or absolute LVEF decline >10% to 50%.
Baseline vs. every 3 months to 15 months after start T	-	CREC definition of cardiotoxicity

Table 4. Continued

Type cardiac biomarker	Study	Number of patients	Chemotherapy
	Onitilo *et al.* (2012) [91]	54	T
	Ky *et al.* (2014) [87]	78	AC + T
CRP	Fallah-rad *et al.* (2011) [89]	42	AC + T
	Morris *et al.* (2011) [93]	59	AC + L + T
MPO	Ky *et al.* (2014) [87]	78	AC + T
	Putt *et al.* (2015) [92]	78	AC + Tax + T
IgE	Beer *et al.* (2016) [102]	7	AC + T
ST2	Sawaya *et al.* (2012) [59]	81	AC + T

Abbreviations: AC, anthracycline + cyclophosphamide; T, trastuzumab; L, lapatinib; Tax, taxanes; C, carboplatin; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; CREC, Cardiac Review and Evaluation Committee.

	Time point(s) indicative of cardiotoxicity	Detection of cardiotoxicity?	Definition cardiotoxicity:
	Maximum values from baseline and every 3 weeks during 1 year T	+	Absolute LVEF decline ≥15% from baseline or an LVEF <50%
	Baseline vs. 3 months after initiation of AC	-	CREC definition of cardiotoxicity
	12 months after initiation of T treatment	-	Absolute LVEF decline >10% to <55% with symptoms of congestive heart failure.
	Baseline	-	Maximal absolute LVEF decline (max LVEF –min LVEF / min LVEF) and congestive heart failure
	Baseline vs. 3 months after initiation of AC	+	CREC definition of cardiotoxicity
	Baseline vs. every 3 months to 15 months after start T	+	CREC definition of cardiotoxicity
	Baseline	+	Absolute LVEF decline ≥10 from baseline to <50%
	Baseline vs. for 1 year follow-up	-	Absolute LVEF decline of ≥5% to <55% with symptoms of heart failure or an asymptomatic absolute LVEF decline ≥10% to <55%

The study of Beer *et al.* showed that high baseline IgE levels were associated with a lower risk of cardiotoxicity in patients treated with anthracycline and trastuzumab.[102] This suggests that the immune system may be a potential mediator in cardiotoxicity caused by anthracycline or trastuzumab. An interesting finding which needs further investigation.

ST2

Suppressor of tumorgenicity 2 (ST2) is a member of the interleukin 1 receptor family. It has a transmembrane (ST2L) and a soluble (sST2) form.[117] As sST2 concentrations reflect cardiovascular stress and myocardial fibrosis, it has become a relative new prognostic biomarker in both acute and chronic heart failure.[118, 119] Combination of ST2 with other cardiac biomarkers may improve the prognostic ability of each individual biomarker in predicting myocardial damage.[120] The 2017 American College of Cardiology/ American Heart Association recommends ST2 in addition to NT-proBNP as biomarker of myocardial fibrosis or injury for additive risk stratification.[121] In a small study of 81 patients, ST2 levels did not change during anthracycline and trastuzumab treatment and measurement of ST2 at completion of anthracycline did not predict subsequent development of cardiotoxicity.[59]

Summary of different biomarkers

Measuring cardiac biomarkers may also be a potential diagnostic tool for early identification of cardiotoxicity. In theory, biomarker monitoring strategies are less expensive, less time-consuming for the patient, easier to perform and may possibly detect myocardial damage at an earlier stage than imaging strategies. However, early identification of patients at high risk for cardiotoxicity with cardiac biomarkers remains questionable and the reason why routine assessment of cardiac biomarkers has not yet been adopted in the daily practice. Taken together, measurement of (a combination of) NT-proBNP, MPO and hs-troponin seems most promising in detecting cardiotoxicity in patients treated with anthracycline and/or trastuzumab. The utility of ST2 and IgE in detecting cardiotoxicity in these patients has to be additionally studied in order to be incorporated into clinical practice. Further research is required to determine the optimal (combination of) biomarker(s), timing of biomarker analysis and the optimal intervention based on these biomarkers results.

Summary and conclusions

Concluding, trastuzumab treatment prolongs progression-free and overall survival in patients with HER2-positive breast cancer but is hampered by cardiotoxicity. Identification of patients at increased risk of trastuzumab-induced cardiotoxicity is of great importance to prevent deterioration to irreversible cardiotoxicity. Nowadays, 3DE seems most

suitable for cardiac monitoring of patients treated with trastuzumab due to the high reproducibility, high accuracy, the ability to assess the complete cardiac structure and the possibility to measure strain. Strain measurement with STE seems promising to detect myocardial damage in an early stage compared to LVEF measurement. However, high quality images and certain technical requirements are needed to perform STE. In addition to STE, early signs of myocardial damage could possibly be detected by measuring cardiac biomarkers of which (a combination of) NT-proBNP, MPO and hs-troponin are most promising. The optimal frequency of cardiac monitoring during trastuzumab therapy has not been established yet. Literature indicates that averagely cardiac monitoring occurs every 3 months during trastuzumab for early-stage breast cancer and more infrequently during trastuzumab for advanced-stage breast cancer. The additional value of frequent cardiac monitoring after discontinuation of trastuzumab has not been confirmed. To validate the role, frequency and duration of STE and certain cardiac biomarkers in clinical practice more and larger studies with clearly defined endpoints in a homogeneous population are urgently awaited.

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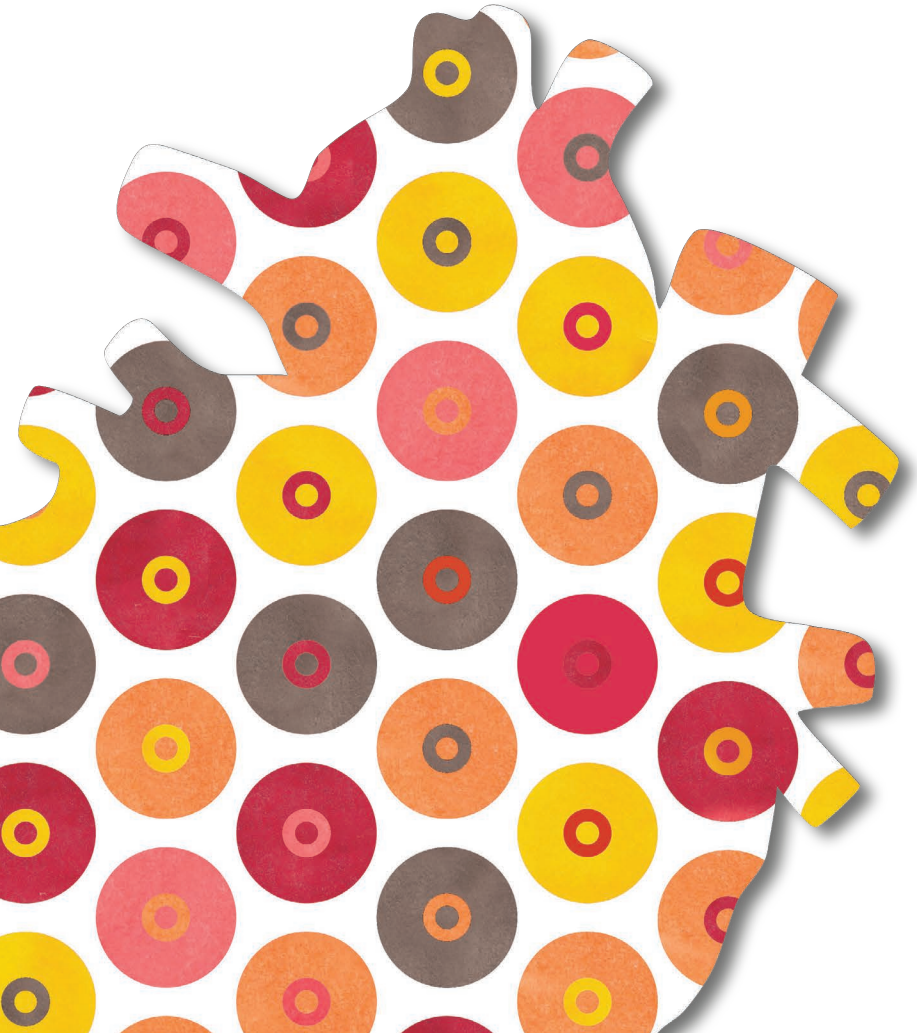

CHAPTER 6

2D-echocardiography versus cardiac MRI strain: a prospective cohort study in patients with HER2-positive breast cancer undergoing trastuzumab

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ABSTRACT

Objective

We aimed to study the predictive value of early two-dimensional echocardiography (2DE) speckle tracking (ST) for left ventricular ejection fraction (LVEF) changes during trastuzumab treatment for HER2-positive breast cancer.

Methods

Patients with HER2-positive breast cancer receiving trastuzumab, with or without anthracycline, underwent 2DE-ST at baseline and after 3 and 6 months trastuzumab. Cardiac magnetic resonance (CMR) imaging (with ST) was performed at baseline and 6 months. We studied the correlation between 2DE-ST- and CMR-derived global longitudinal strain (GLS) and global radial strain (GRS) measured at the same time. Additionally, we associated baseline and 3 months 2DE-ST measurements with later CMR-LVEF, and with cardiotoxicity, defined as CMR-LVEF <45% and/or absolute decline >10% during trastuzumab.

Results

47 patients were included. Median baseline LVEF was 60.4%. GLS measurements based on 2DE-ST and CMR showed weak correlation (Pearson's r=0.33; p=0.041); GRS measurements were uncorrelated (r=0.09; p=0.979). 2DE-LVEF at baseline and 3 months, and 2DE-ST-GLS at 3 months were predictive of CMR-LVEF at 6 months. In contrast, the change in 2DE-ST-GLS at 3 months was predictive of the change in CMR-LVEF at 6 months, whereas the change in 2DE-LVEF was not. Importantly, the 11 patients who developed cardiotoxicity (28%) had larger 2DE-ST-GLS change at 3 months than those who did not (median 5.2%-points versus 1.7%-points; odds ratio for 1% difference change 1.81, 95% confidence interval 1.11 – 2.93; p=0.016; explained variance 0.34).

Conclusions

Correlations between 2DE-ST and CMR-derived measurements are weak. Nevertheless, ST-measurements appeared useful to improve the performance of 2DE in predicting LVEF changes after 6 months of trastuzumab treatment.

BACKGROUND

Patients with HER2-positive breast cancer receiving trastuzumab treatment are prone for developing cardiac dysfunction, which usually represents as a left ventricular ejection fraction (LVEF) decline. Early identification of cardiac dysfunction is important, as further LVEF reductions or development of congestive heart failure may be prevented by cardio-protective treatment with beta-blockers and/or angiotensin converting enzyme (ACE) inhibitors, or by timely interruption of trastuzumab.[1, 2] However, accurate and widely available cardiac monitoring methods are still in development.

Cardiac magnetic resonance (CMR) imaging is the gold standard for evaluation of the cardiac function. CMR has a low inter-reader variability compared to two-dimensional (2DE) or three-dimensional echocardiography (3DE) with respect to LV function and volumes, which is important for serial follow-up.[3] However, the use of CMR for cardiac monitoring of breast cancer patients is hampered by its limited availability and because CMR is experienced by patients as a burdensome procedure. 2DE might be a reasonable, widely available and more readily accepted alternative in this context. Unfortunately, several studies in a variety of patients showed only poor-to-moderate correlation between 2DE and CMR in measuring the LVEF.[4, 5] This could potentially be improved by adding speckle tracking (ST) to 2DE. With 2DE-ST strain imaging can be performed which is a sensitive imaging modality that provides opportunities for detecting subclinical cardiac dysfunction in patients receiving cancer therapy.[6, 7] Although GLS has a moderate intervendor variability, its reproducibility is superior to LVEF measurements and therefore it can be suitable for longitudinal cardiac monitoring.[8, 9]

Studies that investigated 2DE-ST and CMR showed moderate to good correlations ranging from 0.50 to 0.89 for GLS, 0.58 to 0.60 for global radial strain (GRS) and 0.51 to 0.92 for global circumferential strain (GCS) in healthy subjects and in patients with a variety of cardiovascular diseases.[10-17] However, most studies did not differentiate between specific cardiovascular diseases. Subsequently, correlations were not consistent among all subgroups.[17] More importantly, patients who were treated with potential cardio-toxic anti-cancer treatment were not included in these studies. Therefore, validation of these correlations is necessary in these specific populations. Furthermore, a growing number of studies have investigated the clinical relevance of strain measurements in patients during anti-cancer treatment. These studies showed that a GLS decline is related to a LVEF decline measured both with the same methods.[18-21] However, the association between early 2DE-ST strain and later (gold standard) CMR-based LVEF has not been investigated extensively, which is important in determining the additional value of strain imaging along with LVEF evaluation in patients during trastuzumab treatment.

Therefore, the goal of the current study was to investigate the correlation and agreement between 2DE-ST strain and CMR strain, and the association between early 2DE-ST strain measurements and subsequent CMR-derived LVEF in patients with HER2-positive breast cancer during trastuzumab treatment.

METHODS

Study design and participants characteristics

This prospective, observational cohort study included women with HER2-positive early-stage and advanced-stage breast cancer, who underwent trastuzumab treatment from June 2012 until June 2016 in a large teaching hospital in the Netherlands. Patients were excluded from the study in case of baseline CMR-LVEF <45%, ischemic heart disease, valvular heart disease, severe renal dysfunction, hepatic dysfunction or other contraindications for receiving trastuzumab treatment.

In patients with early-stage breast cancer, trastuzumab was preceded by 4 courses of anthracycline. In patients with advanced-stage breast cancer, trastuzumab was administrated once every three weeks until relapse of breast cancer or until the development of cardiotoxicity (for definition see below).[22] The study was approved by the institutional review board of the hospital (WOAC Albert Schweitzer Hospital), and conducted according to the Declaration of Helsinki. All participants provided written informed consent for their participation in the study, and for the study-related measurements.

Echocardiography protocol

2DE was performed at the following time points: before the start of anthracycline (in early-stage patients only), before the start of trastuzumab, after 3-months trastuzumab and after 6 months trastuzumab (Figure 1, Supplementary). 2DE acquisition was performed on a Vivid 7 echocardiography system (GE Vingmed Ultrasound, Trondheim, Norway). End diastolic volume (EDV) and end systolic volume (ESV) were calculated using Simpson's biplane method. The LVEF was determined as the difference between EDV and ESV, relative to the EDV. Baseline measurement were for early-stage patients before the start of anthracycline and for advanced-stage patients before the start of trastuzumab. Strain imaging analyses were then performed using validated tracking algorithm software (TomTec Cardiac Performance Analysis version 4.3 CPA, Unterschliessheim, Germany). EDV and ESV were automatically calculated using traced endocardial borders. These borders that were also used to calculate the GLS and GRS were manually drawn and checked by two experienced observers (Figure 1). GLS was calculated by averaging the

values of peak systolic strain of all 6 segments of the 4-, 3- and 2-chamber views. The shortening of the myocardium related to its original length is described by the negative strain values of GLS. GRS was calculated by averaging the peak systolic strain values in all 6 segments of the parasternal short-axis view at midpapillary level. The thickening of the myocardium is described by the positive strain value of GRS. Treating physicians were blinded for the strain measurements.

CMR imaging protocol

CMR was performed at 2 different time points: before the start of anthracycline (in early-stage patients) or before the start of trastuzumab treatment (advanced-stage patients), and after 6 months trastuzumab treatment in all (Figure 1, Supplementary). CMR examinations were performed with a 1.5-T Achieva Intera scanner (Philips Medical Systems; Best; The Netherlands) applying a standard protocol with validated sequences. Ventricular dimensions and function were assessed with an ECG-gated steady-state free-precession cine MR sequence (echo time, 1.5 to 1.9 ms, repetition time, 2.6 to 3.9 ms; in-plane resolution, 1.5 to 2.0 mm; slice thickness, 4 to 5 mm; number of retrospectively reconstructed images per cardiac cycle, 30). Steady-state free-precession cine imaging sequences were acquired in the ventricular short-axis plane, covering the heart from the plane of the atrioventricular valves through the cardiac apex.

Post-processing CMR software

The artificial intelligence automated CMR software package (Circle Cardiovascular Imaging: cvi version 5.11) applying deep learning was used as post-processing software. Ventricular EDV and ESV were measured using the short-axis stack. LVEF was calculated as the difference between EDV and ESV, relative to the EDV. Endocardial and epicardial contours of the left ventricle that were used for GLS and GRS calculation were automatically tracked using still and motion frames at end-systole and end-diastole (Figure 1). The contours were then checked by two experienced observers, and manually adjusted when necessary. LV contours in the most basal slices were included if >50% of ventricle wall was visible. Additionally, late gadolinium enhancement (LGE) was assessed. This technique incorporates the administration of relatively inert extracellular gadolinium contrast during gradient-echo inversion recovery imaging.

Inter- and intra- observer variability

Inter and intra-observer variability of 2DE-ST and CMR was not assessed as manually traced borders were checked by two experienced observers. Consensus was reached between the two observers regarding the traced borders that were used for GLS and GRS calculation.

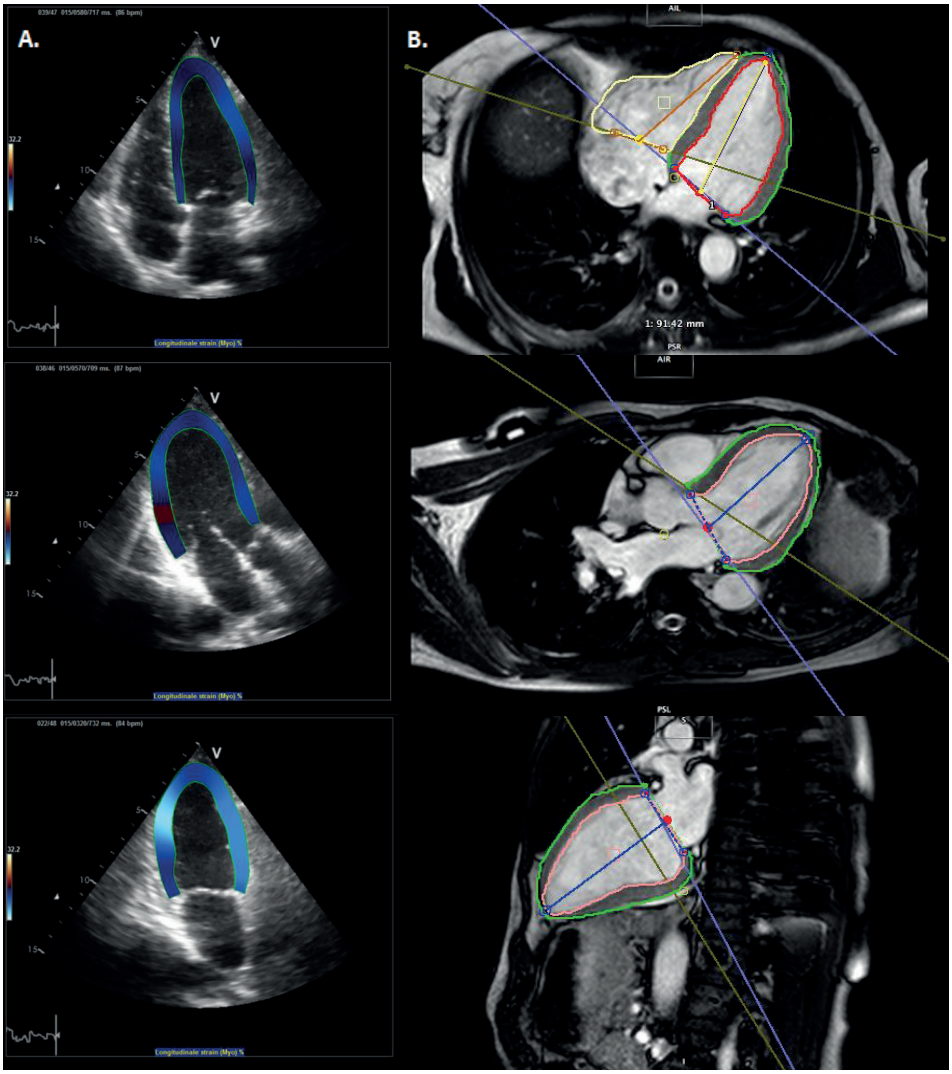

Figure 1. 2D-STE and CMR images used for calculation of myocardial strain

Abbreviations: CMR, cardiac magnetic resonance imaging; 2D-STE, two-dimensional speckle tracking echocardiography.

A. Speckle tracking analysis with 2D-STE of apical 4-chamber, 3-chamber and 2-chamber view.

B. Speckle tracking analysis with CMR of transaxial 4-chamber, 3-chamber and sagittal 2-chamber view.

Cardiotoxicity

Cardiotoxicity was defined as LVEF <45% during the 6 mnths follow-up and/or an absolute LVEF decline of >10% relative to the measurement at study start and measured with CMR – these thresholds are used by the National Cancer Research Institute as definition to interrupt trastuzumab treatment and start ACE inhibitors [23] – and/or any cardiac event for which the patient was hospitalized, including atrial fibrillation, unstable angina pectoris, acute coronary syndrome, and symptomatic heart failure.

Statistical analyses

Categorical baseline data are presented as numbers and percentages. Shapiro-Wilk tests were used to evaluate the normality of continuous baseline data. Normal distributed data were then expressed as mean values ± standard deviation (SD), and non-normal distributed data as median values and interquartile range (IQR).

Nonlinear mixed effects (NLME) models were used to evaluate changes in 2DE-ST and CMR over time. Pearson's correlation coefficients for repeated measurements were determined to assess the correlation between 2DE-ST and CMR. Agreement was assessed with the method of Bland-Altman, likewise using all available repeated measurements. The limits of agreement were defined as the mean difference ± 1.96 SD.

Linear regression analysis was applied to evaluate the association between 2DE-ST strain at different time points and CMR-based LVEF after 6 months trastuzumab treatment. Multivariable linear regression analyses were then applied to evaluate the added value of 2DE-ST strain to 2DE-LVEF measurements on CMR-based LVEF after 6 months trastuzumab treatment. Results of these regression analyses are expressed as the effect on CMR-LVEF per 1 unit difference in the strain value, with its corresponding 95% confidence interval (CI). We also present the corresponding fraction explained variance (R^2).

Logistic regression analysis was used to evaluate the association between 2DE-ST strain at different time points and cardiotoxicity. Results are expressed as odds ratios (ORs) with its corresponding 95% CI.

Data analyses were performed using SPSS software, version 24.0 (SPSS, IBM, Chicago, Illinois, USA) and R statistical software (version 3.4.3), in particular the packages "blandr", "rmcorr" and "lme". Statistical significance of all tests was set at a two-tailed p-value of less than 0.05.

RESULTS

Patients characteristics

A total of 83 patients with HER2-positive breast cancer undergoing trastuzumab treatment signed informed consent for their participation in this study. However, 4 patients only received 1 cycle of trastuzumab, while in 25 patients the baseline CMR remained unperformed, and another 7 had poor 2DE-STE image quality. Hence, 47 patients were available for the current analysis. Median age at inclusion was 57 years (IQR 50, 63 years, Table 1). A total of 38 patients (81%) had early-stage breast cancer and the remaining 9 patients (19%) had advanced-stage breast cancer.

STE measurements

2DE-ST was available for all patients at baseline, for 44 patients (94%) after 3 months trastuzumab treatment and for 42 patients (89%) after 6 months trastuzumab treatment (Table 1, Supplementary). At baseline, median LVEF was 57.2% (IQR 53.3%, 62.6%), GLS -18.8% (-20.6%, -16.3%) and GRS 21.4% (13.5%, 34.1%), respectively (Table 1). During trastuzumab treatment, the mean LVEF declined with -0.47%-points per month (95% CI -0.74%-points, 0.21%-points; p<0.001), whereas the GLS increased with 0.27%-points per month (0.17%-points, 0.38%-points; p<0.001). The mean change in GRS was statistically non-significant (-0.39%-points per month; 95% CI -0.80%-points, 0.03%-points; p=0.070). The course of all 2DE-ST parameters during follow-up are shown in Figure 2.

CMR measurements

CMR images were available for all patients (n=47) at baseline and for 40 patients (85%) after 6 months trastuzumab treatment. At baseline, median CMR-LVEF was 60.4% (IQR 55.8%, 66.0%), GLS -18.7% (-20.1%, -16.9%) and GRS 30.1% (24.5%, 32.9%, Table 1). During trastuzumab treatment, the mean LVEF declined with -0.78%-points per month (95% CI -1.11%-points, -0.44%-points; p<0.001), GLS increased with 0.24%-points per month (95% CI 0.14%-points, 0.32%-points; p<0.001) and GRS declined with -0.68%-points per month (95% CI -0.94%-points, 0.42%-points; p<0.001). No LGE nor edema was observed during trastuzumab treatment. The course of all CMR parameters during follow-up are shown in Figure 2.

Correlations and agreement between 2DE-ST and CMR

For the analysis of the correlation and agreement between 2DE-ST and CMR, a total of 87 combined baseline and 6 months measurements were available. Agreement with respect to LVEF was poor (Figure 3). 2DE-ST-GLS and CMR-GLS showed a significant, but weak correlation (r=0.38; p<0.001). The mean difference was 1.8% (2DE-ST-GLS

-14.7% versus CMR-GLS -16.5%), which was statistically significant (p<0.001). However, the limits of agreement were wide, ranging from -3.9% to 7.5%, suggesting great inter-individual variation. We found no significant correlation for GRS based on both methods (r=0.09; p=0.331) and agreement was poor.

Table 1. Baseline characteristics of the study patients (n=47)

Age, years	57.0 (50.0, 63.0)
	55.0 (10.1)
BMI, kg/m²	24.5 (23.1, 29.4)
	25.9 (4.8)
Breast cancer	
Early-stage	38 (81)
Advanced-stage	9 (19)
Anthracycline-based chemotherapy	38 (81)
Left-sided radiotherapy	12 (26)
Cardiovascular risk factors	
Hypertension	17 (36)
Diabetes mellitus	3 (6)
Hypercholesterolemia	7 (15)
Positive family history	15 (32)
Current or ever smoker	13 (28)
Cardiac condition before treatment	
Valve insuffiency	0 (0)
Arrhytmia	1 (2)
MI/CABG/PCI	0 (0)
Cardiovascular medication	
Beta-blockers	1 (2)
ACE inhibitors	2 (4)
Both	1 (2)
CMR imaging parameters	
LVEF, %	60.4 (55.8, 66.0)
	60.6 (7.3)
GLS, %	-18.7 (-20.1, -16.9)
	-18.1 (5.6)

Table 1. Continued

GRS, %	30.1 (24.5, 32.9)
	29.5 (5.8)
Left ventricular mass, g	73.6 (65.4, 88.0)
	76.3 (15.2)
Length left ventricle diastolic phase, mm	86.0 (81.5, 89.5)
	85.9 (6.6)
LGE, %	6.0 (5.0 – 8.0)
	6.9 (3.1)
2DE parameters	
LVEF, %	66.0 (63.0, 73.0)
	67.5 (6.5)
ST-LVEF, %	57.2 (53.3, 62.6)
	56.9 (8.1)
ST-GLS, %	-18.8 (-20.6, -16.3)
	-18.4 (3.0)
ST-GRS, %	21.4 (13.5, 34.1)
	23.6 (13.1)

All continuous variables are shown as median + IQR, mean + SD.
Abbreviations: BMI, body mass index; MI, myocardial infarction; CABG, coronary arterial bypass grafting; PCI, percutaneous coronary intervention; ACE, angiotensin converting enzyme; CMR, cardiac magnetic resonance imaging; 2DE, two-dimensional echocardiography; LVEF, left ventricular ejection fraction; GLS, global longitudinal strain; GRS, global radial strain; LGE, late gadolinium enhancement.

Predictive value of 2DE-ST strain for CMR-based LVEF and cardiotoxicity

Table 2 presents the relations between early 2DE-ST measurements and later CMR-LVEF. 2DE-LVEF before start of trastuzumab and after 3 months trastuzumab treatment were predictive of CMR-LVEF at 6 months trastuzumab treatment. For example, a 1%-points difference in 2DE-LVEF before start of trastuzumab was related with a mean difference of 0.85%-points in CMR-LVEF at 6 months trastuzumab treatment (95% CI 0.42%-points, 1.27%-points; $p<0.001$). Early 2DE-LVEF values during anthracycline treatment, as well as change values at 3 months trastuzumab treatment, failed to predict CMR-LVEF changes.

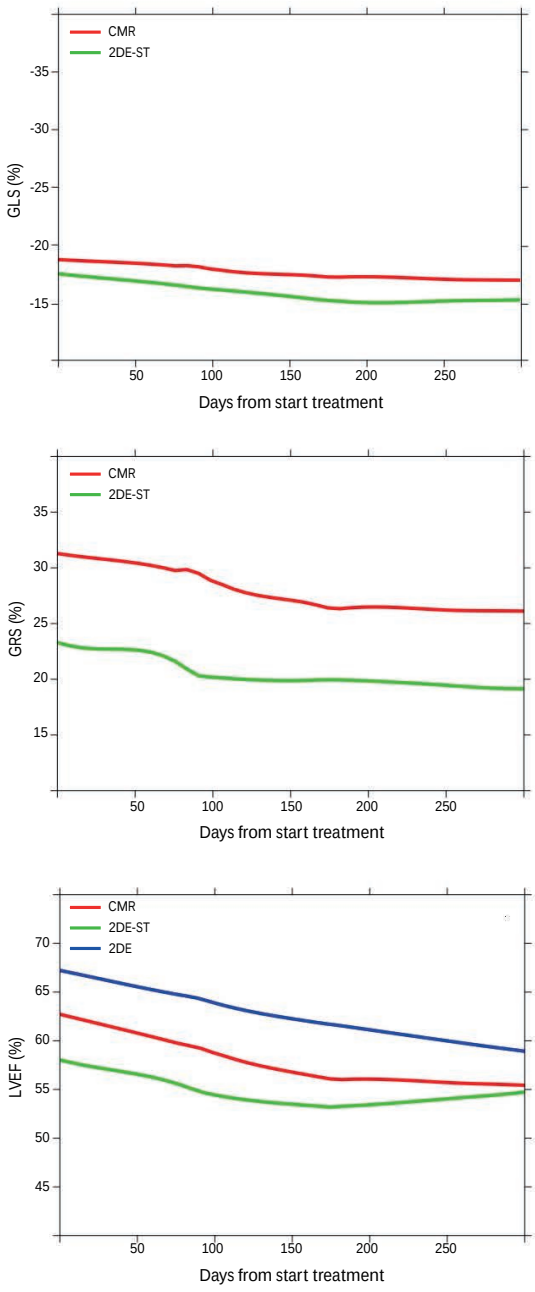

Figure 2. Temporal evolution of 2DE-ST, 2DE and CMR parameters during follow-up

Abbreviations: 2DE-ST, two-dimensional speckle tracking echocardiography; CMR, cardiac magnetic resonance imaging; LVEF, left ventricular ejection fraction; GLS, global longitudinal strain; GRS, global radial strain; 2DE, two-dimensional echocardiography.

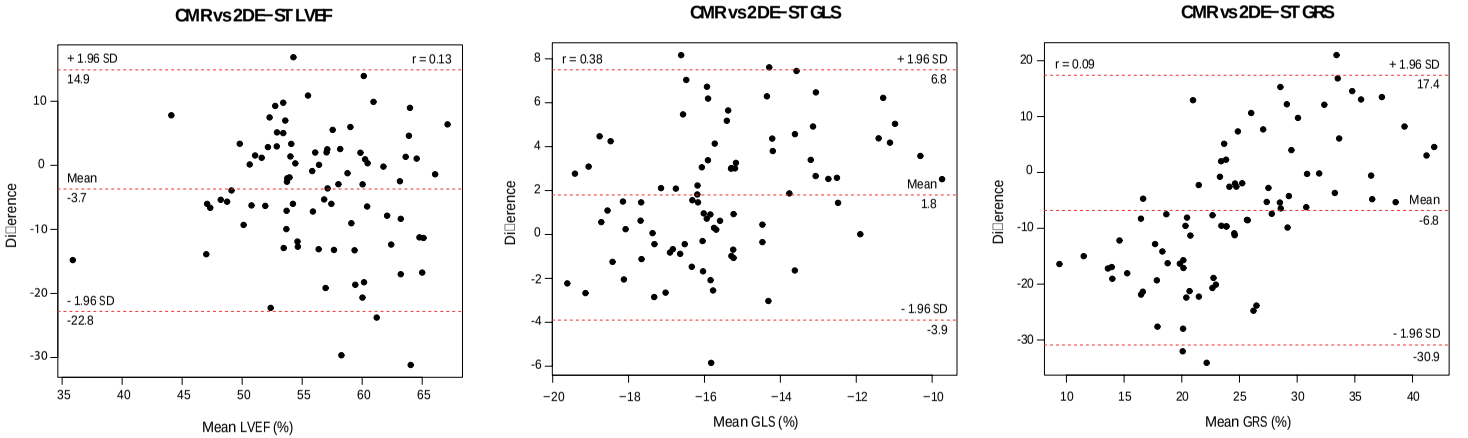

Figure 3. Correlation and agreement between CMR and 2DE-ST

Difference was calculated as 2DE-ST minus CMR.

Abbreviations: CMR, cardiac magnetic resonance imaging; 2DE-ST, two-dimensional speckle tracking echocardiography; LVEF, left ventricular ejection fraction; GLS, global longitudinal strain; GRS, global radial strain.

Patients with higher 2DE-ST-GLS at 3 months trastuzumab treatment demonstrated significantly lower CMR-LVEF at 6 months trastuzumab treatment, but significance was lost after adjustment for 2DE-LVEF. In contrast to 2DE-LVEF, 2DE-ST-GLS change values at 3 months trastuzumab treatment were predictive of CMR-LVEF changes at 6 months trastuzumab treatment. Sensitivity analyses in patients with early-stage breast cancer and advanced-stage breast cancer showed similar results (Table 2, Supplementary)

Importantly, 11 patients (28%) developed cardiotoxicity, of whom all experienced an absolute LVEF decline >10%-point from baseline and 3 patients additionally reached an LVEF below 45%. These patients who developed cardiotoxicity had a median GLS of -15.2% at baseline, which was not statistically different from the median GLS of -16.8% at baseline of patients who did not developed cardiotoxicity (p=0.674). In addition, a larger 2DE-GLS change at 3 months trastuzumab treatment was observed in those who developed cardiotoxicity than in those who did not (median 5.2%-points versus 1.7%-points, p=0.036, Table 2). The odds ratio for a 1%-point difference in change was 1.81 (95% CI 1.11, 2.93; p=0.016). The explained variance of the latter model was 0.34, indicating a moderate effect. Finally, the trajectory of GLS of patients with and without cardiotoxicity showed a trend for a higher GLS increase per month in patients with cardiotoxicity compared to patients without cardiotoxicity (median 0.65%-points versus 0.20%-points, p=0.181, Figure 4).

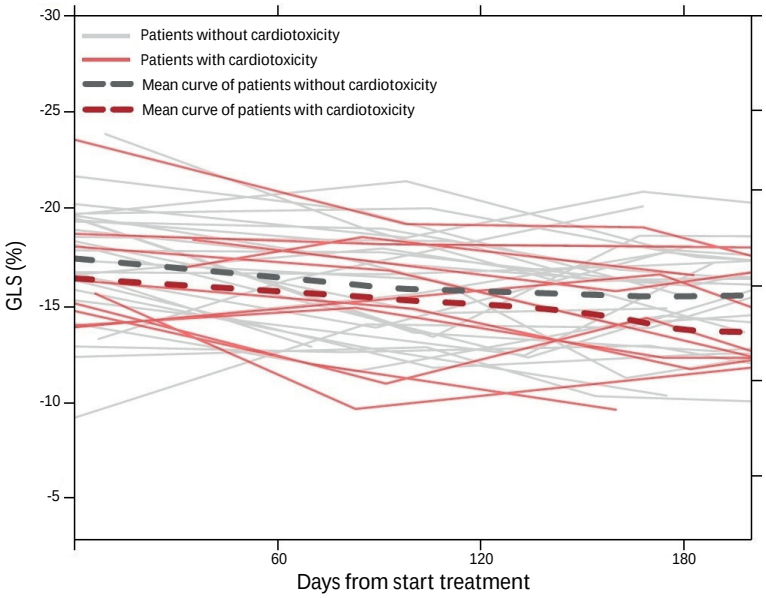

Figure 4. Trajectory of GLS of patients with and without cardiotoxicity

Abbreviations: GLS; global longitudinal strain.

Table 2. Association between measures obtained by 2DE-ST before anthracycline, before trastuzumab and after 3 months trastuzumab, and CMR-LVEF at 6 mont

2DE	**CMR-LVEF at 6 months after start trastuzumab**					
	Univariable analysis			**Multivariable analysis**		
	Mean difference (95% CI)	**P-value**	**R^2**	**Mean difference (95% CI)**	**P-value**	**R^2**
Before start anthracycline						
LVEF, %	0.42 (-0.02, 0.85)	0.058	0.11			
ST-GLS, %	0.24 (-0.83, 1.31)	0.649	0.01			
ST-GRS, %	0.11 (-0.11, 0.33)	0.322	0.01			
Change during anthracycline						
LVEF, %	0.11 (-0.29, 0.51)	0.570	0.01	0.03 (-0.39, 0.45)	0.879	0.07
ST-GLS, %	-0.86 (-2.02, 0.30)	0.142	0.07	-0.83 (-2.08, 0.42)	0.186	
ST-GRS, %	-0.04 (-0.39, 0.31)	0.829	0.00			
Before start trastuzumab						
LVEF, %	0.85 (0.42, 1.27)	<0.001	0.30	0.87 (0.41, 1.34)	<0.001	0.30
ST-GLS, %	-0.42 (-1.31, 0.46)	0.337	0.03	0.14 (-0.68, 0.95)	0.738	
ST-GRS, %	0.08 (-0.13, 0.28)	0.464	0.07			
3 Months after start trastuzumab						
LVEF, %	0.59 (0.30, 0.88)	<0.001	0.32	0.56 (0.24, 0.87)	0.001	0.35
ST-GLS, %	-1.14 (-2.07, -0.19)	0.018	0.10	-0.46 (-1.34, 0.41)	0.288	
ST-GRS, %	0.04 (-0.23, 0.31)	0.751	0.00			
Change at 3 months after start trastuzumab						
LVEF, %	0.30 (-0.11, -0.71)	0.144	0.06	0.33 (-0.06, 0.72)	0.094	0.22
ST-GLS, %	-1.17 (-2.14, -0.20)	0.019	0.14	-1.20 (-2.16, -0.24)	0.016	
ST-GRS, %	-0.17 (-0.57, 0.23)	0.386	0.03			

Abbreviations: 2DE, two-dimensional echocardiography; CMR, cardiac magnetic resonance imaging; GLS, global longitudinal strain; GRS, global radial strain; LVEF, left ventricular ejection fraction; OR, odds ratio; R^2, explained variance; ST, speckle tracking; CTOX, cardiotoxicity.

Change in CMR-LVEF at 6 months after start trastuzumab			Cardiotoxicity[a]				
Univariable				Univariable			
Mean difference (95% CI)	P-value	R^2	Median in patients with CTOX [IQR]	median in patients without CTOX [IQR]	OR (95% CI)	P-value	R^2
0.23 (-0.22, 0.68)	0.297	0.04	63.0 [58.8, 65.5]	69.0 [64.0, 77.0]	0.77 (0.61, 0.97)	0.027	0.26
0.42 (-0.64, 1.49)	0.422	0.02	-17.0 [-18.5, -14.6]	-16.0 [-18.5, -13.0]	0.81 (0.59, 1.11)	0.377	0.05
-0.03 (-0.26, 0.19)	0.767	0.09	26.5 [15.2, 36.0]	27.2 [13.4, 36.5]	0.99 (0.93, 1.05)	0.721	0.01
-0.05 (-0.45, 0.35)	0.790	0.01	-2.0 [-7.0, 1.0]	-7.0 [-13.0, 0.0]	1.07 (0.95, 1.21)	0.250	0.05
-0.86 (-2.03, 0.30)	0.141	0.07	4.4 [0.0, 6.0]	0.2 [-0.1, 2.0]	1.39 (0.99, 1.95)	0.058	0.12
0.08 (-0.27, 0.43)	0.631	0.01	-4.2 [-14.0, 2.3]	-0.9 [-12.2, 4.3]	1.00 (0.92, 1.10)	0.942	0.00
0.32 (-0.16, 0.80)	0.189	0.05	60.0 [57.3, 62.8]	63.0 [60.0, 66.0]	0.88 (0.75, 1.02)	0.080	0.13
-0.28 (-1.14, 0.58)	0.519	0.01	-13.7 [-16.9, -11.2]	-15.0 [-17.3, -12.2]	1.13 (0.87, 1.46)	0.365	0.03
-0.02 (-0.22, 0.18)	0.831	0.00	31.4 [4.5, 42.1]	22.4 [8.5, 35.5]	0.99 (0.94, 1.05)	0.830	0.00
0.29 (-0.04, 0.61)	0.080	0.08	55.0 [43.4, 62.8]	60.0 [57.0, 63.0]	0.85 (0.74, 0.98)	0.029	0.28
-0.62 (-1.54, 0.30)	0.179	0.05	-11.6 [-15.3, -9.4]	-14.1 [-16.6, -11.7]	1.36 (0.94, 1.84)	0.073	0.13
-0.15 (-0.40, 0.10)	0.237	0.04	28.1 [8.5, 35.3]	21.2 [12.1, 32.0]	1.03 (0.95, 1.11)	0.532	0.02
0.21 (-0.19, 0.61)	0.292	0.03	-11.0 [-19.0, -2.0]	-4.0 [-7.0, 0.0]	0.90 (0.80, 1.01)	0.079	0.09
-1.10 (-2.02, -0.18)	0.021	0.14	5.2 [2.8, 7.0]	1.7 [-0.2, 3.0]	1.81 (1.11, 2.93)	0.016	0.34
-0.12 (-0.52, 0.27)	0.521	0.02	-0.7 [-10.0, 2.0]	-3.0 [-9.7, 1.4]	1.03 (0.92, 1.15)	0.610	0.01

[a] Cardiotoxicity was defined as LVEF <45% during the 6 months follow-up and/or an absolute LVEF decline of >10% relative to the measurement at study start and measured with CMR.

DISCUSSION

In a broad range of clinical practices, 2DE remains the most obvious imaging modality for the evaluation of therapy-related cardiotoxicity in oncology patients.[24] Nevertheless, 2DE only has a moderate to poor agreement with gold standard CMR regarding the evaluation left ventricular function.[4] We demonstrated that speckle tracking improved the performance of 2DE to predict LVEF changes in HER2-positive breast cancer patients receiving trastuzumab. In particular detrimental, cardiotoxic changes could be predicted with greater accuracy, although there is room for further improvement.

Studies on the correlation between 2DE-GLS and CMR-GLS, and 2DE-ST LVEF and CMR-LVEF showed a wide variation.[10-17, 25] Reported correlation coefficients range from 0.16 in a series of 10 heart transplant recipients to 0.89 in a similar small number of patients with aortic valve stenosis (Table 3, Supplementary). In general, correlation analyses in this field are hampered by small sample sizes, so that estimates are surrounded by uncertainty. That aside, it seems that stronger correlations are reported by studies that included heterogeneous populations of patients undergoing 'clinically indicated' echocardiography or CMR, who agreed to undergo the other imaging modality too.[14, 15] Some of these studies even combine observations in patients and healthy volunteers.[10, 11, 17] In general, weaker correlations are reported in studies that focussed on specific, homogeneous populations (including ours). It is well-known that spurious(ly strong) correlations can occur when groups are pooled with differences in absolute values of the variable of interest.[26] For example, Amzulescu *et al*. reported a high intraclass correlation coefficient (ICC) of 0.89 in a combined series of healthy volunteers (mean 2DE-GLS -21%), and patients with aortic stenosis (mean 2DE-GLS -18%), hypertrophic cardiomyopathy (mean 2DE-GLS -15%), ischemic heart disease (mean 2DE-GLS -14%) or non-ischemic dilated cardiomyopathy (mean 2DE-GLS -12%), whereas correlations in the separate subgroups were less convincing.[10] We believe that individual-patient meta-analyses of available datasets are warranted to obtain reliable estimates in relevant target groups. Such analyses are also useful to study reported inter-software variability with respect to strain calculations in more detail.[27]

We found only a very weak correlation between 2DE and CMR with respect to GRS. Indeed, in most studies, correlations for GRS were weaker than for GLS (Table 3, Supplementary).[14, 17] This might be due to the difficulty of epicardial border tracking in 2DE images, and due to the fact that apical views are more suitable for tracking speckles in the longitudinal direction, than in the radial direction.[28] Additionally, a trend to lower 2DE-ST measured LVEF was observed to compared 2DE-LVEF (Figure 2). This can be explained by differences between the two techniques leading to an underestimation

of the LVEF measured with 2DE-ST. Underestimation of the LVEF by 2DE-ST has been previously described when comparing 2DE-ST with 3DE, although an explanation is still missing.[29] Therefore, additional research is necessary.

Interestingly, the subgroup analysis in patients without prior anthracycline exposure showed that early GLS change was not associated with CMR-LVEF at 6 months or a change in CMR-LVEF at 6 months (Table S2, Supplementary). This could be explained by the fact that non-anthracycline based trastuzumab treatment is associated with much lower cardiotoxicity (cardiotoxicity incidence of 3-7% versus 27%).[30] As prior anthracycline exposure is an important risk factor for developing trastuzumab-induced cardiotoxicity [31, 32], it might be useful to consider including only patients with prior anthracycline exposure before trastuzumab treatment for future studies.

Our observation that a GLS decline measured with 2DE is related to a subsequent lower CMR-based LVEF (and cardiotoxicity) corresponds with previous studies and meta-analyses.[18-21, 33] Hence, change values appear to contain prognostic information. Accordingly, the American Society of Echocardiography (ASE) and European Association of Cardiovascular Imaging (EACVI) recommends that acquired GLS during chemotherapy should be compared with baseline values.[7] Based on the results of our study, it seems reasonable to add speckle tracking to the 2DE protocol for the regular cardiac surveillance of cancer patients before and during trastuzumab therapy. Importantly, the same modality should be used for serial cardiac surveillance to avoid pitfalls introduced by limited agreement between the modalities.[7] In our follow-up scheme that is based on the current guideline for cardiac monitoring of HER2-positive breast cancer patients during adjuvant or metastatic trastuzumab treatment [34], an abnormal GLS measured with 2DE preceded a LVEF decline by about 3 months. This may provide a window of opportunity to start early cardio-protective therapy. In a small series of HER2-positive breast cancer patients, the recently SAFE-HEART study confirmed that trastuzumab can be safely continued in those with compromised cardiac function, provided that cardiac treatment is timely installed.[35] Recently, the 1 year-results of the prospective multicenter SUCCOUR trial showed that a GLS-guided cardio-protective treatment strategy reduced the incidence of cardiotoxicity, defined as LVEF decline >10% to <55%, compared to a LVEF-guided cardio-protective treatment strategy (5.8% versus 13.7%, p=0.02).[36]

Finally, 3DE-ST may potentially have superior tracking quality over 2DE-ST, as speckles can be tracked in all possible directions and through-plane motion will be absent. It is true that several studies report stronger correlations for GLS and GRS between 3DE-ST and CMR than 2DE-ST and CMR. (11, 14) However, aside from the fact that these studies studied heterogeneous populations, which hampers the interpretation of the findings, it

must be realized that the accuracy of 3DE-ST strongly depends on operator experience (26), more so than with 2DE-ST. Unfortunately, in this study we were unable to perform 3DE-ST to study the correlations with CMR in this specific population. Additional studies with larger numbers of participants are required before this technique can be implemented into daily clinical practice.

Limitations

Several limitations have to be taken into account when interpreting our results. First, we performed a single-center study. Although this center is representative for large, secondary, teaching hospitals, we were unable to study external validity of our findings. Secondly, the sample size was small, although similar to other studies in the field. Consequently, the power was limited to study the additive predictive value of 2DE-derived GLS and LVEF in greater detail. In addition, due to the small numbers of cardiotoxicity events (n=11), multivariable modelling of predictors for cardiotoxicity was not possible. Lastly, most patients included in our study were diagnosed with early-stage breast cancer for which they were treated with anthracycline and sequential trastuzumab. As these patients only received CMR before anthracycline and not before trastuzumab treatment, we could not investigate the effect of strain on the LVEF change during trastuzumab treatment only. Sensitivity analyses in the early-stage breast cancer patients and advanced-stage breast cancer patients showed, despite the small numbers, similar results.

Conclusions

In our series of patients with HER2-positive breast cancer with preserved left ventricular function prior to trastuzumab treatment, correlations between 2DE-ST and CMR-derived measurements were weak. Nevertheless, speckle tracking appeared to be useful to improve the performance of 2DE to predict detrimental LVEF changes during 6 months trastuzumab treatment, but much remains to be done.

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SUPPLEMENTAL MATERIAL
CHAPTER 6

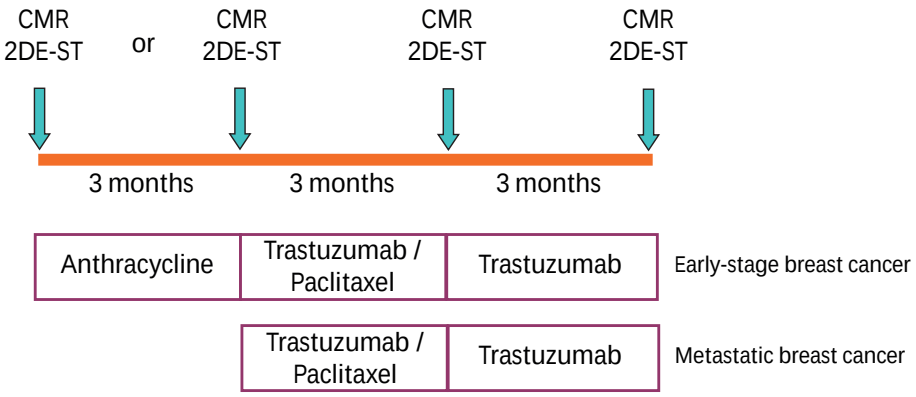


Figure S1. Study procedures: participants were studied before chemotherapy and before start trastuzumab at standardized intervals every 12 weeks using echocardiography.

Abbreviations: CMR, cardiac magnetic resonance imaging; 2D-STE, two-dimensional speckle tracking echocardiography.

Table S1. All parameters during follow-up

Before anthracycline (n)
LVEF, %
GLS, %
GRS, %
Before start trastuzumab (n)
LVEF, %
GLS, %
GRS, %
3 months after start trastuzumab (n)
LVEF, %
GLS, %
GRS, %
6 months after start trastuzumab (n)
LVEF, %
GLS, %
GRS, %
Absolute change baseline – 3 months (n)
LVEF, %
GLS, %
GRS, %
Absolute change baseline – 6 months (n)
LVEF, %
GLS, %
GRS, %

Abbreviations: 2DE-ST; two-dimensional speckle tracking echocardiography, CMR; cardiac magnetic resonance imaging, LVEF; left ventricular ejection fraction, GLS; global longitudinal strain, GRS; global radial strain, 2DE; two-dimensional echocardiography, NA; not applicable.

CMR	2DE-ST	2DE
Median (IQR)	**Median (IQR)**	**Median (IQR)**
38	38	37
60.5 (55.0, 66.1)	59.6 (53.5, 64.7)	66.0 (63.0, 75.0)
-17.0 (-18.5, -14.9)	-19.0 (-20.7, -16.4)	
30.9 (24.5, 34.4)	20.8 (14.2, 33.8)	
9	47	41
59.4 (56.7, 66.5)	55.9 (51.0, 59.2)	62.5 (58.3, 66.0)
-16.6 (-17.6, -14.9)	-16.7 (-18.3, -13.9)	
27.2 (23.9, 30.5)	23.0 (9.1, 34.3)	
NA	44	42
	51.8 (48.5, 56.0)	59.5 (56.0, 63.0)
	-14.8 (-16.7, -11.7)	
	16.4 (9.9 to 30.5)	
40	42	44
54.8 (50.7, 60.6)	54.7 (49.7, 58.2)	60.0 (55.0, 64.0)
-16.1 (-17.3, -14.4)	-14.9 (-16.9, -12.5)	
26.7 (22.5, 28.9)	19.0 (12.0, 27.2)	
NA	44	42
	-4.8 (-10.3, 0.4)	-8.0 (-2.0, -14.0)
	3.5 (1.5 to 4.9)	
	-3.7 (-9.7, 1.4)	
40	42	44
-5.4 (-10.0, -0.2)	-2.7 (-11.3, 3.8)	-6.0 (-0.5, -13.0)
1.5 (0.3, 2.1)	3.3 (1.0, 5.6)	
-4.5 (-7.5, -0.6)	-3.0 (11.7, 3.0)	

Table S2. Sensitivity analyses in early-stage breast and advanced-stage breast cancer

2DE	CMR-LVEF at 6 months		
	Univariable analysis		
	mean difference (95% CI)	P-value	R^2
Before start anthracycline			
Early-stage patients (n=38)			
LVEF, %	0.42 (-0.02, 0.85)	0.058	0.11
ST-GLS, %	0.24 (-0.83, 1.31)	0.649	0.01
ST-GRS, %	0.11 (-0.11, 0.33)	0.322	0.01
Before start trastuzumab			
Early-stage patients (n=38)			
LVEF, %	0.79 (0.34, 1.25)	0.001	0.29
ST-GLS, %	-0.49 (-1.47, 0.48)	0.311	0.03
ST-GRS, %	0.09 (-0.12, 0.31)	0.386	0.03
Advanced-stage patients (n=8)			
LVEF, %	0.91 (-0.63, 2.46)	0.199	0.26
ST-GLS, %	0.52 (-2.33, 3.38)	0.669	0.03
ST-GRS, %	0.06 (-0.79, 0.91)	0.869	0.01
3 Months after start trastuzumab			
Early-stage patients (n=38)			
LVEF, %	0.52 (0.17, 0.88)	0.006	0.24
ST-GLS, %	-1.29 (-2.25, -0.33)	0.010	0.21
ST-GRS, %	0.06 (-0.23, 0.36	0.664	0.01
Advanced-stage patients (n=8)			
LVEF, %	0.76 (-0.03, 1.55)	0.058	0.48
ST-GLS, %	1.33 (-1.70, 4.37)	0.324	0.16
ST-GRS, %	0.25 (-0.70, 1.20)	0.545	0.06
Change during anthracycline			
Early-stage patients (n=38)			
LVEF, %	0.11 (-0.29, 0.51)	0.570	0.01
ST-GLS, %	-0.86 (-2.02, 0.30)	0.142	0.07
ST-GRS, %	-0.04 (-0.39, 0.31)	0.829	0.00
Change at 3 months after start trastuzumab			

	CMR-LVEF at 6 months			Change in CMR-LVEF at 6 months		
	Multivariable analysis			Univariable		
	mean difference (95% CI)	P-value	R^2	mean difference (95% CI)	P-value	R^2
				0.23 (-0.22, 0.68)	0.297	0.04
				0.42 (-0.64, 1.49)	0.422	0.02
				-0.03 (-0.26, 0.19)	0.767	0.09
	0.85 (0.33, 1.37)	0.002	0.30	0.23 (-0.30, 0.77)	0.385	0.03
	0.22 (-0.74, 1.17)	0.641		-0.26 (-1.25, 0.72)	0.588	0.01
				0.00 (-0.21, 0.22)	0.979	0.00
	0.89 (-0.93, 2.70)	0.266	0.26	0.42 (-0.88, 1.71)	0.464	0.09
	0.22 (-2.71, 3.15)	0.853		0.55 (-1.59, 2.68)	0.555	0.06
				-0.08 (-0.72, 0.57)	0.782	0.01
	0.40 (-0.01, 0.81)	0.057	0.31	0.21 (-0.19, 0.61)	0.297	0.04
	-0.85 (-1.91, 0.20)	0.109		-0.84 (-1.84, 0.16)	0.097	0.10
				-0.14 (-0.43, 0.14)	0.314	0.04
	0.70 (-0.31, 1.70)	0.133	0.49	0.19 (-0.62, 1.00)	0.587	0.05
	0.40 (2.65, 3.44)	0.751		1.05 (-1.24, 3.34)	0.304	0.17
				0.12 (-0.62, 0.85)	0.716	0.02
	0.03 (-0.39, 0.45)	0.879	0.07	-0.05 (-0.45, 0.35)	0.790	0.00
	-0.83 (-2.08, 0.43)	0.186		-0.86 (-2.03, 0.30)	0.141	0.07
				0.08 (-0.27, 0.43)	0.631	0.01

Table S2. Continued

2DE	CMR-LVEF at 6 months		
	Univariable analysis		
	mean difference (95% CI)	P-value	R^2
Early-stage patients (n=38)			
LVEF, %	0.13 (-0.34, 0.60)	0.538	0.01
ST-GLS, %	--1.55 (-2.53, 0.56)	0.003	0.27
ST-GRS, %	-0.18 (-0.57, 0.22)	0.367	0.03
Advanced-stage patients (n=8)			
LVEF, %	0.69 (-0.54, 1.91)	0.219	0.24
ST-GLS, %	0.60 (-2.53, 3.74)	0.655	0.04
ST-GRS, %	0.32 (-1.00, 1.64)	0.573	0.06
Change from start anthracycline to 3 months after start trastuzumab			
Early-stage patients (n=38)			
LVEF, %	0.21 (-0.20, 0.63)	0.303	0.04
ST-GLS, %	-1.54 (-2.54, -0.54)	0.004	0.27
ST-GRS, %	-0.17 (-0.57, 0.23)	0.386	0.03

Abbreviations: CMR, cardiac magnetic resonance imaging; AC, anthracycline; 2DE-ST, two-dimensional speckle tracking echocardiography; GLS, global longitudinal strain; GRS, global radial strain; 2DE, two-dimensional echocardiography; LVEF, left ventricular ejection fraction; CI, confidence interval, R^2: fraction explained variance.

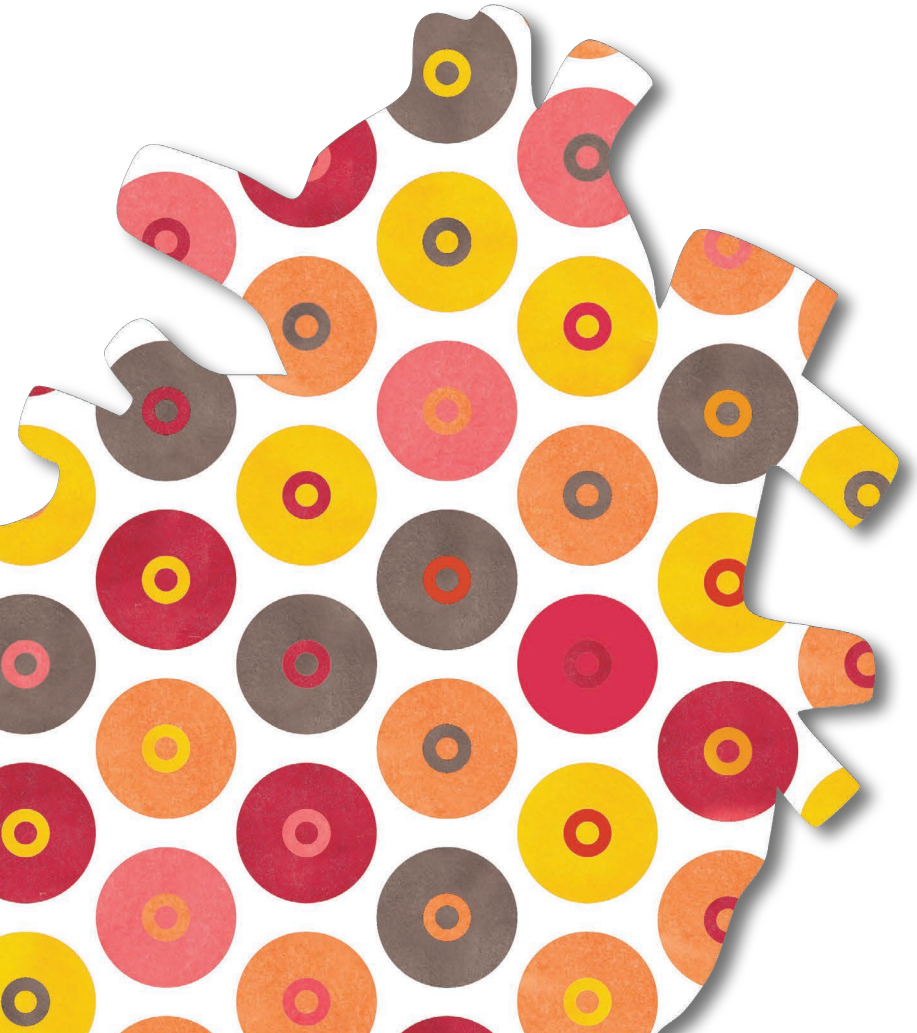
	CMR-LVEF at 6 months			Change in CMR-LVEF at 6 months		
	Multivariable analysis			Univariable		
	mean difference (95% CI)	P-value	R^2	mean difference (95% CI)	P-value	R^2
	0.13 (-0.29, 0.56)	0.526	0.28	0.12 (-0.35, 0.59)	0.608	0.01
	-1.54 (-2.55, -0.52)	0.005		-1.37 (-2.37, -0.38)	0.009	0.22
				-0.13 (-0.51, 0.26)	0.510	0.02
	0.69 (-0.84, 2.22)	0.300	0.24	0.06 (-1.00, 1.12)	0.898	0.00
	-0.02 (-3.52, 3.47)	0.988		0.31 (-2.09, 2.71)	0.761	0.02
				0.41 (-0.53, 1.35)	0.327	0.02
	0.07 (-0.34, 0.47)	0.737	0.27	0.03 (-0.39, 0.45)	0.894	0.00
	-1.49 (-2.56, -0.41)	0.009		-1.37 (-2.38, -0.35)	0.010	0.22
				-0.12 (-0.52, 0.27)	0.521	0.02

Table S3. Overview of studies investigating the correlation of CMR and 2D-STE in measuring strain

Study	Population	
Obokata *et al.* (2015)	Clinically indicated CMR	
Pryds *et al.* (2019)	Healthy subjects	(n=10)
	HFrEF	(n=10) (n=10)
	Aortic valve stenosis	(n=10)
	Heart transplantation	
	Perimyocarditis	
Erley *et al.* (2019)	Ischemic heart disease	(n=15)
	Non-ischemic heart disease	(n=33)
	Clinically indicated CMR	(n=2)
Amzulescu *et al.* (2017)	Healthy volunteers	(n=31)
	Ischemic heart disease	(n=39)
	Non-ischemic heart disease	(n=36)
	Hypertrophic cardiomyopathy	(n=11)
	Aortic stenosis	(n=19)
Amzulescu *et al.* (2018)	Healthy volunteers	(n=29)
	LV dysfunction	(n=63)
	LV hypertrophy	(n=29)
Onishi *et al.* (2015)	Clinically indicated CMR	
Kaku *et al.* (2014)	Clinically indicated CMR	
Cho *et al.* (2006)	Ischemic heart disease	
Bansal *et al.* (2008)	Ischemic heart disease	

Abbreviations: GLS, global longitudinal strain; GRS, global radial strain; GCS, global circumferential strain; CMR, cardiac magnetic resonance imaging; CMR-FT, cardiac MRI feature tracking; 2DE-ST, two-dimensional speckle tracking echocardiography; 3DE-ST, three-dimensional speckle tracking echocardiography; HFrEF, heart failure with reduced ejection fraction; r, Pearson's R; ICC, intraclass correlations; LV, left ventricular.

Sample size	Correlation GLS GRS GCS	Compared techniques
106	r=0.83 r=0.69 r=0.90 r=0.87 r=0.82 r=0.88	2DE-ST vs. CMR-FT 3DE-ST vs. CMR-FT
50	Overall r=0.74 r=0.58 r=0.76 Healthy subjects r=0.32 r=0.43 r=0.17 HFrEF r=0.16 r=0.12 r=-0.1 Perimyocarditis r=0.87 r=0.08 r=0.80 Aortic valve stenosis r=0.89 r=0.86 r=0.90 Heart transplantation r=0.15 r=0.41 r=0.79	2DE-ST vs. CMR-FT
50	r=0.71	2DE-ST vs. CMR-FT
136	ICC=0.89 ICC=0.80	2DE-ST vs. CMR tagging
119	ICC=0.65 ICC=0.55 ICC=0.89 ICC=0.83	2DE-ST vs. CMR tagging 3DE-ST vs. CMR tagging
73	r=-0.87 r=-0.92	2DE-ST vs. CMR-FT
19	r=0.87 r=0.61 r=0.78	3DE-ST vs. CMR
30	r=0.51 r=0.60 r=0.51	2DE-ST vs. CMR tagging
30	r=0.50 r=0.59 r=0.63	2DE-ST vs. CMR tagging



CHAPTER 7

NT-proBNP correlates with LVEF decline in HER2-positive breast cancer patients treated with trastuzumab

Nathalie I Bouwer, Crista Liesting, Marcel JM Kofflard, Sylvia M Sprangers-van Campen, Jasper J Brugts, Jos JEM Kitzen, Michael A Fouraux, Mark-David Levin, Eric Boersma

Cardio-Oncology. 2019; 5:4.

ABSTRACT

Background
Early identification of cardiac dysfunction by non-invasive imaging in patients with HER2-positive breast cancer treated with trastuzumab is challenging. In particular multigated acquisition (MUGA) scanning, which is most widely used, is unable to detect subclinical cardiac changes. The use of N-terminal pro-brain natriuretic peptide (NT-proBNP), a serum biomarker of myocardial stress, might improve timely diagnosis.

Methods
This prospective, single-center, cohort study included patients with HER2-positive breast cancer who started trastuzumab treatment. Echocardiography was scheduled at regular intervals every 3 months during one year follow-up for cardiac function monitoring. For research purposes, NT-proBNP was determined at the same time points. Trastuzumab-induced cardiotoxicity (TIC) was the primary study endpoint, defined as a left ventricular ejection fraction (LVEF) <45%, and/or an absolute decline in LVEF >10% since inclusion, and/or the incidence of a clinical cardiac event including atrial fibrillation, unstable angina pectoris, acute coronary syndrome, and symptomatic heart failure.

Results
A total of 135 patients were enrolled between January 2008 and June 2016, with a median age of 54 years (IQR 47 – 61 years). By three-dimensional echocardiography (3DE), the median LVEF at baseline was 62% (IQR 58 - 65%). At a median of 6 months (IQR 5 – 11 months), 45 patients (33%) reached the study endpoint of TIC. Patients with TIC had a mean change of -9.5% in LVEF (95% CI -7.2 to -11.7; p=0.001) during 1 year of trastuzumab treatment. Both NT-proBNP at baseline (HR 1.04, 95% CI 1.02 – 1.07; p=0.003) and LVEF decline during anthracycline treatment prior to the start of trastuzumab (HR 1.16, 95%CI 1.07 – 1.25; p<0.001) were independently associated with development of TIC. The level of NT-proBNP during follow-up was associated too with development of TIC (HR 1.06 per 10 pmol/l difference, 95% CI 1.02 – 1.10; p=0.008). No steadily or sudden increase in NT-proBNP prior to TIC was observed.

Conclusions
NT-proBNP cannot be used as a surrogate monitoring tool for trastuzumab-induced cardiotoxicity in HER2-positive breast cancer patients during the first year of treatment. Patients showing an LVEF decline during anthracycline pre-treatment appeared vulnerable for trastuzumab-induced cardiotoxicity.

INTRODUCTION

The identification of cardiac dysfunction in patients with HER2-positive breast cancer treated with trastuzumab is challenging, but crucial in order to prevent the development of heart failure in these patients. Trastuzumab (Herceptin®, Genetech, San Francisco CA) is a highly effective anti-cancer drug that is widely used in patients with HER2-positive breast cancer. Addition of trastuzumab to neoadjuvant or adjuvant chemotherapy in patients with HER2-positive breast cancer improved disease-free survival (DFS) and overall survival (OS) impressively.[1-3] However, trastuzumab may cause cardiotoxicity, foremost an impairment of the left ventricular ejection fraction (LVEF), which may adversely affect the prognosis and limit quality of life.

In clinical practice, it is essential to identify early subclinical cardiac dysfunction in breast cancer patients treated with trastuzumab. Clinical symptomatic heart failure might than be prevented by timely prescription of cardio-protective medication, or by interruption or even discontinuation of trastuzumab treatment, as trastuzumab-induced cardiotoxicity might be (partially) reversible.[4] Multigated acquisition (MUGA) scans are widely used to monitor cardiac function in this patient population, but identification of cardiotoxicity by this technique is challenging. MUGA scans not only provides LVEF assessments with high inter- and intra-observer variability,[5] but also fail to detect early subclinical cardiac alterations, because of initial compensatory mechanisms of the left ventricle to prevent functional cardiac impairment.[6] In addition, cardiac monitoring by serial MUGA scans will pose a high radiation burden to the patient.[7] Echocardiography, another frequently used cardiac monitoring approach, overcomes certain limitations of MUGA scans as echocardiography evaluates the complete cardiac structure and lacks radiation exposure. Therefore, more advanced and sensitive diagnostic strategies are needed, and cardiac biomarkers, including N-terminal pro-brain natriuretic peptide (NT-proBNP) might be useful in this respect.

NT-proBNP is a peptide stored in, and secreted predominantly from, membrane granules in the ventricles of the heart in response to increased intra cardiac pressure.[8] NT-proBNP is an established serum biomarker for the diagnosis of heart failure [9], and is associated with adverse prognosis in heart failure patients. As trastuzumab-based therapy may induce ventricle wall stress, small changes in NT-proBNP levels can potentially be detected prior to an LVEF decline. Studies investigating the association between NT-proBNP levels and cardiotoxicity in breast cancer patients showed inconclusive results.[10-17] Two large prospective studies demonstrated increased NT-proBNP levels in breast cancer patients with cardiotoxicity [14, 17], but several others failed.[10-13, 15, 16] These studies are hampered by a low incidence of LVEF declines compared with population based, retrospective studies (9% vs. 19%) or predominant focus on anthracyclines instead of trastuzumab. [18, 19]

The current, prospective cohort study was designed to overcome these limitations. We aimed to assess the potency of a screening-strategy utilizing repeatedly measured NT-proBNP levels to detect trastuzumab-induced cardiotoxicity measured with three-dimensional echocardiography (3DE) in a representative cohort of patients with HER2-positive breast cancer.

METHODS

Selection and description of participants

This prospective cohort study included women with HER2-positive breast cancer, who started trastuzumab treatment between April 2008 and June 2016 in the Albert Schweitzer Hospital (ASZ), a large teaching hospital in Dordrecht, the Netherlands.

Patients were excluded from the study in case of baseline LVEF <45%, presence of cardiac dysfunction, ischemic heart disease, valvular heart disease, severe renal dysfunction or hepatic dysfunction or known intolerability for trastuzumab treatment. This study was approved by the institutional review board of the ASZ and was conducted according to the Declaration of Helsinki. All participants provided written informed consent.

Procedures

Indications for trastuzumab treatment included neoadjuvant or adjuvant (early-stage) and metastatic (advanced-stage) breast cancer with overexpression of the HER2. Patients were treated according to prevailing guidelines.[20] In patients with early-stage breast cancer, trastuzumab was preceded by 4 courses of doxorubicin 50-60 mg/m^2 and cyclophosphamide 500-600 mg/m^2 once every 3 weeks. After these 4 courses, trastuzumab 2-4 mg/kg was administered in combination with paclitaxel 80 mg/m^2 weekly for 12 cycles, followed by monotherapy trastuzumab 6 mg/kg every three weeks for one year. In patients with advanced-stage breast cancer, 6 courses of trastuzumab 2-4 mg/kg in combination with paclitaxel 80 mg/m^2 were administered as initial treatment, after which trastuzumab 6 mg/kg was continued once every three weeks until relapse of breast cancer or until the development of cardiotoxicity or other reasons to stop trastuzumab.[21] In this study, patients were studied during the first year of trastuzumab treatment.

Echocardiography and laboratory assessments

Echocardiography and laboratory assessments were scheduled during the first year of follow-up (Supplementary Figure S1). Within two weeks before or after blood collection, 3DE was systematically performed, with exclusion of eight days after the first trastuzumab

administration (Supplementary Figure S1). Although MUGA scan is currently most used in monitoring the cardiac function in these patients, ECG-gated triplane 3DE was used as routine cardiac monitoring because of the high accuracy, lack of radiation exposure, comprehensive evaluation of cardiac structure and reproducibility.[5] No MUGA scans were performed during the year of follow-up.

The 3DE images were obtained using a 1.5 to 3.6 MHz 3V probe with the Vivid 7 echo system (GE Vingmed Ultrasound, Trondheim, Norway). Gain and compression were set at 50%, and harmonic imaging was also used. Data sets were acquired from the parasternal long-axis, apical and subcostal position. Left ventricle (LV) volumes and LVEF were measured offline by dedicated software (Echopac). The data set was aligned in 2 orthogonal planes along the long axis of the LV with clear depiction of the mitral valve and the LV apex. End diastolic measurements corresponded with the largest chamber size, whereas end systolic measurements corresponded with the smallest chamber size. The LVEF was determined as the difference between end diastolic volume (EDV) and end systolic volume (ESV), relative to the EDV. The echocardiograms were recorded by two independent experts at the echo core laboratory and evaluated by one cardiologist for research objectives.

Venous blood was systematically collected at the following time points: immediately before the start of anthracycline treatment (in early-stage patients only), immediately before the start of trastuzumab treatment, eight days after the start of trastuzumab and subsequently once every 12 weeks until 48 weeks after start of trastuzumab (Supplementary Figure S1). At each time point, the NT-proBNP level (Dimension Vista 500, Siemens Healthcare Diagnostics, Deerfield, Illinois) was measured according to the manufacturer's instructions. Critical value for the NT-proBNP assay was <10%. Treating physicians were not aware of the patients' NT-proBNP levels.

Study endpoints

The primary endpoint of the study was the occurrence of trastuzumab-induced cardiotoxicity (TIC), which was defined as an LVEF <45% at any scheduled follow-up time point and/or an absolute decline in LVEF of >10% relative to the measurement at study start – these thresholds are used by the National Cancer Research Institute as definition to interrupt trastuzumab treatment and start ACE inhibitors [22] – and/or any cardiac event for which the patient was hospitalized, including atrial fibrillation, unstable angina pectoris, acute coronary syndrome, and symptomatic heart failure. Patients who died in the year of follow up were censored at the last available 3DE date.

Statistical analyses

Categorical baseline data are presented as numbers and percentages. Normality of continuous baseline data was evaluated by Shapiro-Wilk tests. Normal distributed data are presented as mean values ± one standard deviation (SD), and non-normal distributed data as median values and interquartile range (IQR).

Cox proportional hazard analysis was used to analyze the relation between the following baseline characteristics and the development of TIC: age at diagnosis of breast cancer, LVEF at baseline, decline in LVEF during anthracycline treatment, and NT-proBNP at baseline. The decline in LVEF during anthracycline treatment was calculated as the difference between the LVEF at start trastuzumab and at start anthracycline treatment.

Linear mixed effect models (LMM) were used to study the LVEF and NT-proBNP change over time, as well as the relation between repeatedly measured NT-proBNP and LVEF. Echocardiography and blood collection was not necessarily performed at the same date. For this analysis, practically, we considered the closest NT-proBNP measurement within 30 days before or after an LVEF measurement as the corresponding value.

Joint modeling (JM) was applied to study the relation of repeatedly measured NT-proBNP with the incidence of TIC during follow-up. The JM combined a LMM model, describing the temporal evolution of NT-proBNP, with a Cox proportional hazard regress model, describing the time-to-event process.

Data analyses were performed using SPSS software, version 24.0 (SPSS, IBM, Chicago, Illinois, USA) and R statistical software (version 3.4.3), in particular the packages "LME" and "JM". Statistical significance of all tests was set at a two-tailed p-value of less than 0.05.

RESULTS

Patient characteristics

Between April 2008 and June 2016, 150 patients with HER2-positive breast cancer were enrolled. A total of 15 patients were excluded from the analyses because they did not receive trastuzumab treatment (n=4), had no echocardiography (n=8) or NT-proBNP measurement (n=3). Hence, a total of 135 patients were available for analyses (Figure 1). Median age of the patients at inclusion was 54 years (IQR 47 – 61; Table 1). Of all included patients, 113 patients (84%) had early-stage breast cancer, whereas the remaining patients had advanced-stage disease. Prior cardiac disease including valve insufficiency, arrhythmia and myocardial ischemia was present at start of treatment in 10 of the included patients (7%).

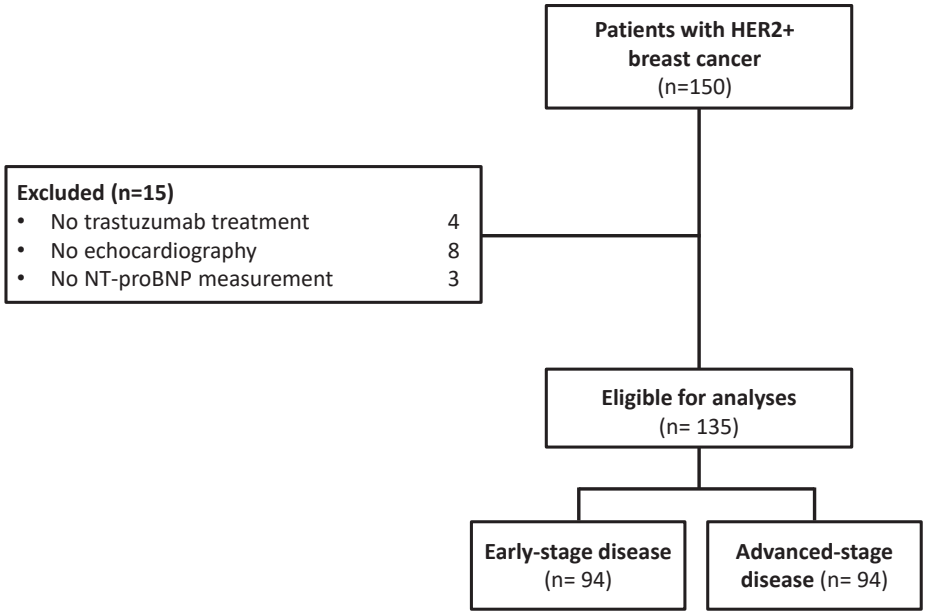


Figure 1. Flowchart of patient inclusion

Abbreviations: HER2+, Human Epidermal growth factor Receptor positive; NT-proBNP, N-terminal pro-brain natriuretic peptide.

Trastuzumab-induced cardiotoxicity

During the 1 year of follow-up, 4 patients (3%) died. All patients died because of disease progression (Table 2). The range of the last available LVEFs of these patients was 53% to 62%.

In total, 45 patients (33%) developed TIC during treatment with trastuzumab (Table 2), of whom 44 patients (98%) experienced an absolute LVEF decline of >10%. A total of 16 patients (36%) displayed an LVEF <45% during trastuzumab treatment. We found no difference in TIC between patients with early-stage disease and advanced-stage disease (p=0.805). The median time to TIC was 6 months (IQR 5 – 11 months). Two patients developed diastolic dysfunction grade 2 or higher. These patients also demonstrated TIC. No other clinical cardiac events were observed.

The treating physician postponed treatment with trastuzumab in 6 patients temporarily and in 14 patients permanently because of cardiotoxicity. LVEF improved and returned to normal after discontinuation of trastuzumab treatment in all but 2 patients (10%).

Table 1. Characteristics of included patients (n=135)

Characteristics
Age (years)
BMI (kg/m^2)
Duration of trastuzumab treatment (months)
Pretreatment with anthracycline (doxorubicin)
Local radiotherapy
Cardiac condition before treatment • Valve insufficiency • Arrhythmia • MI/CABG/PCI
LVEF (%)
NT-proBNP (pmol/l)[c]
Follow-up duration (months)[d]

Abbreviations: BMI, body mass index; MI, myocardial infarction; CABG, coronary artery bypass graft; PCI, percutaneous coronary intervention; LVEF, left ventricle ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; IQR, interquartile range.

Table 2. Clinical outcomes during 1 year of trastuzumab treatment

Clinical outcomes
Cardiac events
Trastuzumab-induced cardiotoxicity
Solely LVEF <45%
Solely absolute LVEF decline>10% from baseline
LVEF <45% and absolute LVEF decline >10% baseline
Total[a]
Atrial fibrillation
Myocardial ischemia
Other cardiac events
Diastolic dysfunction grade 3 or 4
Postponement of trastuzumab
Temporarily
Permanent
Death
Progression disease

Abbreviations: 3DE, three-dimensional echocardiography; LVEF, left ventricle ejection fraction.
[a] LVEF <45% and/or decline >10%.

	Total (n=135) No. (%), median [IQR]	**Early-stage (n=113)** No. (%), median [IQR]	**Advanced-stage (n=22)** No. (%), median [IQR]
	54 [47 - 61]	53 [47 – 60]	61 [53 – 65]
	25.6 [23.7 – 29.5]	25.6 [23.6 – 29.4]	26.1 [23.9 – 30.1]
	11 [11 – 12]	11 [11 – 11]	16 [9 – 44]
	111 (82)	107 (95)	4 (18)
	61 (45)	53 (47)	8 (36)
	5 (3.5)	3 (2.6)	2 (9)
	5 (3.7)	4 (3.5%)	1 (4.5)
	0	0	0
	62 [58 – 65]	62 [58 – 65][a]	61 [57 – 66][b]
	9 [5 – 14]	8 [5 – 14]	11 [7 – 18]
	13 [11 – 14]	13 [11 – 14]	11 [9 – 12]

[a] Measured at T0 with 3DE.

[b] Measured at T1 with 3DE.

[c] Measured at baseline.

[d] Calculated from start anthracycline treatment to last available LVEF or NT-proBNP measurement.

	Total (n=135), No. (%)	**Early-stage (n=113),** No. (%)	**Advanced-stage (n=22),** No. (%)
	1 (1)	0	1 (5)
	29 (21)	26 (23)	3 (14)
	15 (11)	12 (11)	3 (14)
	45 (33)	38 (34)	7 (32)
	0	0	0
	0	0	0
	0	0	0
	2 (2)	1 (1)	1 (5)
	6 (4)	6 (5)	0
	14 (10)	12 (11)	2 (9)
	4 (3)	2 (2)	2 (9)

Baseline factors associated with TIC

Age at diagnosis of breast cancer and baseline LVEF were not associated with TIC during 1 year of trastuzumab treatment (Table 3). However, patients who developed TIC showed an LVEF change of -6.6% during anthracycline treatment prior to the start of trastuzumab versus an LVEF change of -0.8% in patients without TIC (p=0.033). The hazard ratio (HR) of an anthracycline-induced LVEF decline for TIC was 1.16 (95% CI 1.07 – 1.25, p<0.001). Although patients with TIC did not have significant higher baseline NT-proBNP than patients who remained TIC-free (median of 12 pmol/l versus 8 pmol/l, mean difference of 4 pmol/l, p=0.229), single measurement of NT-proBNP at baseline was related with the development of TIC during follow-up (HR 1.04, 95% CI 1.02 – 1.07, p=0.003).

Temporal evolution of LVEF

A total of 770 3DEs were obtained, of which 9 could not be interpreted because of poor quality, leaving 761 available for analysis, which implies a median of 6 LVEF measurements (IQR 5 – 6 measurements) per patient. The median LVEF at baseline was 61% (IQR 59 - 65%).

In all patients together, during 1 year of trastuzumab treatment, the mean LVEF declined by 4.5% (95% CI -3.3% to -5.8%; p<0.001). In fact, this was mainly driven by the patients with TIC, who showed a change of -9.5% in LVEF (95% CI -7.2% to -11.7%; p=0.001) as compared to a change of -1.6% in LVEF (95%CI -0.6% to -2.7%; p=0.944) in their TIC-free counterparts. A post-hoc analysis of the 107 early-stage disease patients receiving anthracycline treatment demonstrated that, when comparing LVEF before anthracycline treatment with the LVEF at end of anthracycline treatment, patients with TIC experienced an LVEF decline of 0.056% per day and patients without TIC an LVEF decline of 0.002% per day. Figure 2 shows the trajectory of LVEF of patients with and without TIC in patients with and without anthracycline pretreatment.

Temporal evolution of NT-proBNP

A total of 692 NT-proBNP values were determined with a median of 6 NT-proBNP measurements per patient (IQR 4 – 7 measurements). NT-proBNP and LVEF were related, and every +10 pmol/l difference in NT-proBNP (at any time point during follow-up) was associated with an absolute difference in LVEF of -4.5% (95% CI -2.2% to -6.7%; p<0.001). Mean levels of NT-proBNP in patients with and without TIC were 16.8 and 10.1 pmol/l, respectively, which implies a mean difference of 6.7 pmol/l (p=0.031). The HR for developing TIC was 1.06 per +10 pmol/l difference in NT-proBNP at any time point during follow-up (95% CI 1.02 – 1.10, p=0.008). NT-proBNP levels in all individual patients slightly increased from baseline (+2.9 pmol/l), more so in patients with TIC (+10.2 pmol/l) than in those without (+2.5 pmol/l), and this difference was statistically significant (p=0.037). Interestingly, there was no evidence of a steadily or sudden increase in NT-proBNP prior to TIC. (Figure 3)

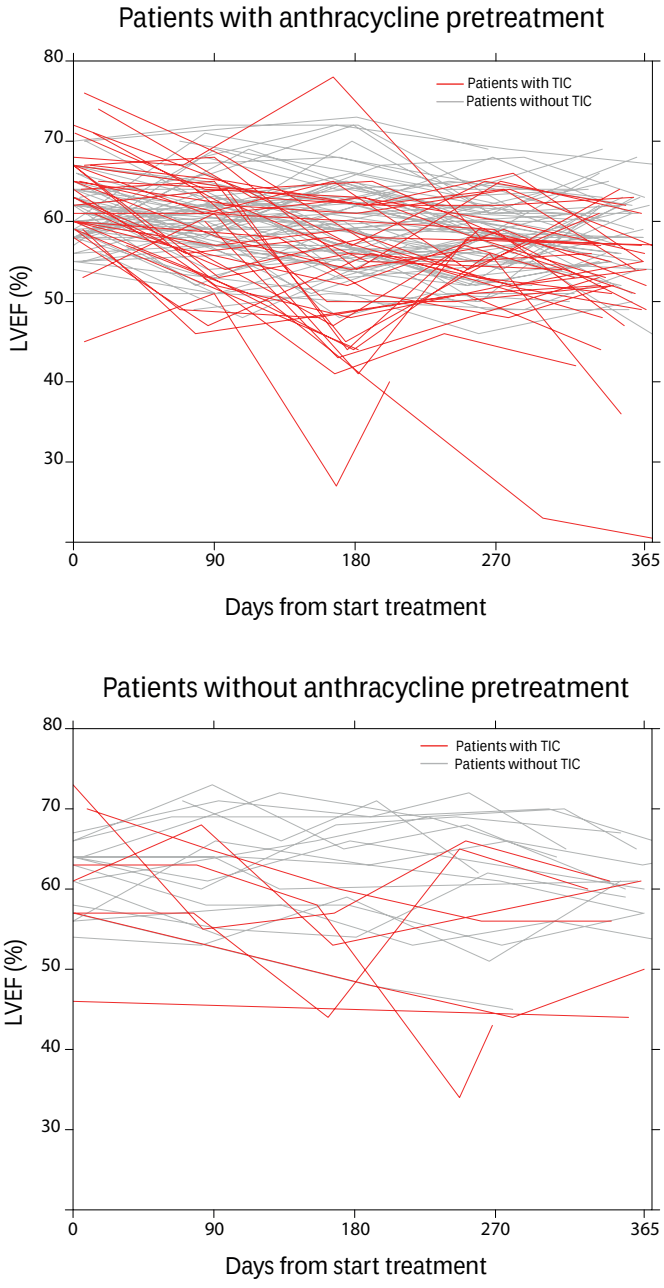


Figure 2. Trajectory of LVEF and NT-proBNP of patients with TIC and without in patients with and without anthracycline pretreatment

Abbreviations: LVEF, left ventricle ejection fraction; TIC, trastuzumab-induced cardiotoxicity; NT-proBNP, N-terminal pro-brain natriuretic peptide.

Table 3. Risk factors associated with TIC during 1 year of trastuzumab treatment

Independent variables	Adjusted HR	95% CI
NT-proBNP (pmol/l)[b]	1.04	1.02 – 1.07
LVEF (%)[b]	1.06	0.98 – 1.15
LVEF decline (%) during anthracycline treatment	1.16	1.07 – 1.25
Age (years)[c]	0.99	0.96 – 1.02

Abbreviations: NT-proBNP, N-terminal pro-brain natriuretic peptide; LVEF, left ventricle ejection fraction; HR, hazard ratio; TIC, trastuzumab-induced cardiotoxicity.

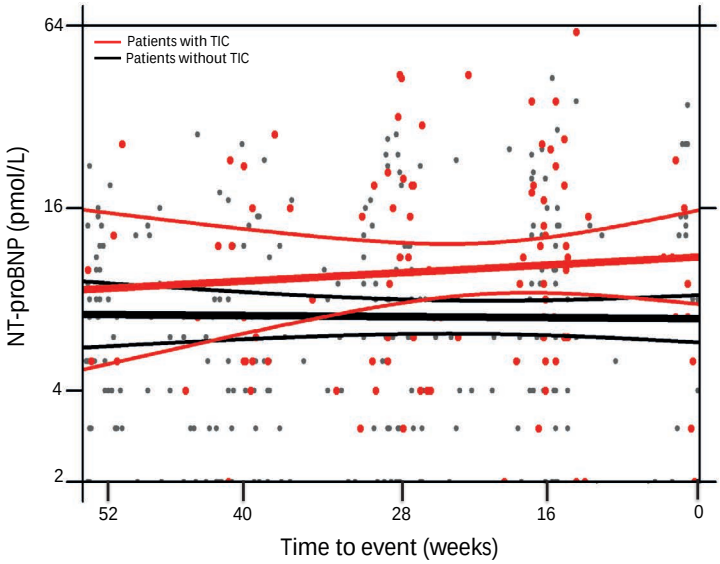


Figure 3. Trajectory of NT-proBNP before TIC or last follow-up of patients without TIC

Abbreviations: NT-proBNP, N-terminal pro-brain natriuretic peptide; TIC, trastuzumab-induced cardiotoxicity.

DISCUSSION

This study in patients with HER2-positive breast cancer showed that serum levels of NT-proBNP increased during the first year of trastuzumab treatment. Patients who developed cardiotoxicity showed a steeper increase and had on average higher NT-proBNP levels than those in whom their left ventricular function remained preserved. Still, NT-proBNP failed as a biomarker for early identification of cardiac dysfunction, as the changes were too subtle and could barely be distinguished from normal intra-subject variability.[23]

P-value	Patients with TIC Median [IQR]	Patients without TIC Median [IQR]	P-value[a]
0.003	12 [5 – 19]	8 [5 – 12]	0.229
0.136	64 [60 – 67]	60 [58 – 63]	0.003
<0.001	6.6 [2 – 9]	0.8 [-3 – 4]	0.033
0.513	53 [47 – 61]	55 [47 – 61]	0.296

[a] P-value obtained from comparison of values patients with and without TIC.
[b] Measured at baseline.

Our observation that NT-proBNP increases during trastuzumab treatment was also described by the studies of Romano *et al.* and Zardavas *et al.*[14, 17] Romano *et al.* also demonstrated that an increase of NT-proBNP during anthracycline is predictive for cardiotoxicity within 3 to 12 months.[14] This is in contrast with various other studies, that neither found increased NT-proBNP values during trastuzumab treatment, nor a relation between NT-proBNP and the incidence of cardiotoxicity. [10-13, 15, 16] The explanation of these variable results is most likely multifactorial, and related to the type of treatment, sample size, the specific NT-proBNP assay used, and, probably most relevant, the definition of cardiotoxicity (Supplementary Table S1). In the current study, a clinically relevant and widely accepted international definition of cardiotoxicity was used (LVEF decline of >10% and/or LVEF <45%). We opted for this strict definition, as patients will usually only start to experience cardiac symptoms when lower LVEF levels are reached. In addition, an LVEF decline of >10% can suggest an increased risk of heart failure and cardio-protective treatment is advised. It should also be noted that in the study of Romano *et al.*, patients exclusively received anthracycline-based chemotherapy, and trastuzumab-based chemotherapy was not administered.[14]

NT-proBNP for early identification of cardiotoxicity

No evidence was found of a steadily or sudden increase in NT-proBNP before the development of TIC. Therefore, we concluded that NT-proBNP is not suited for the early identification of TIC. Still, in the current study, NT-proBNP did increase during trastuzumab treatment, and this increase was indeed related with a declining LVEF. The question on the role of NT-proBNP in detecting cardiotoxicity in patients receiving trastuzumab therefore still remains. The intra- and inter-subject variability of NT-proBNP are noteworthy, and the types of NT-proBNP assays used in the studies differ. To overcome these drawbacks, further research regarding NT-proBNP should be considered in larger, international, multi-center studies.

Whether cardiac biomarkers in general are suitable for detecting cardiotoxicity in patients receiving cardio-toxic cancer treatment also remains questionable. There are multiple pathways that could be involved in the development of cardiotoxicity due to cardio-toxic cancer treatment, and therefore multiple biomarkers may be useful in detecting cardiotoxicity. The pathway of anthracycline involves inhibition of topoisomerase IIb in myocardial cells with apoptosis and radical oxygen species formation as result [24], but less is known about the specific pathway involved in trastuzumab-induced cardiotoxicity. For example, troponin is released in patients with ischemic heart disease, but until now it has not been proven efficient in detecting cardiotoxicity due to trastuzumab treatment.[10, 12, 13, 25, 26] More knowledge about the pathway of trastuzumab-induced cardiotoxicity could possibly identify (new) cardiac biomarkers which could be useful for detecting this cardiotoxicity. Recently, the prognostic value of the biomarker suppressor of tumorgenicity 2 (ST2) became evident for acute [27] and chronic [28] heart failure patients. However, this biomarker, alone or in combinations with other cardiac biomarkers, has not been investigated extensively in patients undergoing cardio-toxic chemotherapy and/or immunotherapy for HER2-positive breast cancer.

Role of imaging technique in detection of cardiotoxicity

Three-dimensional echocardiography, not standard MUGA scan, was used in the current study for the reason that 3DE is the preferred technique for LVEF monitoring and detection of cardiotoxicity due to the high accuracy in detecting LVEF levels below the lower limit of normal, high reproducibility and low temporal variability.[5] Major limitations of measuring LVEF with MUGA-scans are the questionable accuracy, the cumulative radiation exposure of serial monitoring, intra-and inter variability and its limited information on other cardiac structures.[5, 7, 29-31] It should be noted that the choice of imaging modality can influence the estimated incidence of cardiotoxicity, and thus the relation with NT-proBNP. The other studies investigating the relationship between NT-proBNP and cardiotoxicity used two-dimensional echocardiography (2DE) for the LVEF assessments. However, 2DE is suggested to overestimate the mean LVEF by 5%.[32] Because 3DE-LVEF measurements do not systematically differ from cardiac MRI (CMR) LVEF measurements, the gold standard for LVEF measurements, and 3DE has a higher availability than CMR, we used 3DE in this study.[32] However, it cannot be completely excluded that some of our results represent false-positive results.

Interestingly, our study showed that patients with a (steeper than average) decrease in LVEF after anthracycline-based chemotherapy are most likely to develop cardiotoxicity during trastuzumab-based treatment. Thus, importantly, these patients can be identified prior to the start of trastuzumab, as they can potentially benefit from strict cardiac monitoring. This is in line with the European Society for Medical Oncology (ESMO) guidelines that recommend

to reassess the LVEF if during anthracycline-based chemotherapy the LVEF declines below the 50% and to consider therapy for left ventricular dysfunction if the LVEF is confirmed to be below the 50% after anthracycline-based chemotherapy.[33] More should be learned about which factors - the cardiac response to anthracycline treatment, or the cardiac response to trastuzumab treatment, or others - are most important regarding the development of cardiotoxicity during trastuzumab treatment.

Limitations

Several limitations were inherent to the study design and limit the interpretation of our findings. First, all patients were treated and monitored in one single hospital. Although this hospital is a large, secondary, teaching-hospital in the Netherlands, it must be taken into account that external validity of the study findings had not been demonstrated. Second, our sample size was possibly too small in regard of the intra-subject variability of both NT-proBNP and LVEF. Although, compared with similar studies on the topic, our study had one of the highest number of included patients (Supplementary Table S1). Third, in some cases there was a time delay between the NT-proBNP measurement and 3DE at one time point. Although we only observed a steady and slow increase in NT-proBNP coupled with a steady decrease in LVEF, the timing mismatch might have influenced our findings. At last, the echocardiograms were made by two different persons and reviewed by one cardiologist, inter-observer variability in the LVEF results cannot completely be ruled out. However, 3DE is known to have a low inter-observer variability of 0.027% compared to 2DE.[34]

Conclusion

NT-proBNP cannot be used as a surrogate monitoring tool for cardiotoxicity of trastuzumab in HER2-positive breast cancer patients during the first year of treatment. Patients showing an LVEF decline during anthracycline pre-treatment appeared vulnerable for trastuzumab-induced cardiotoxicity.

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SUPPLEMENTAL MATERIAL
CHAPTER 7

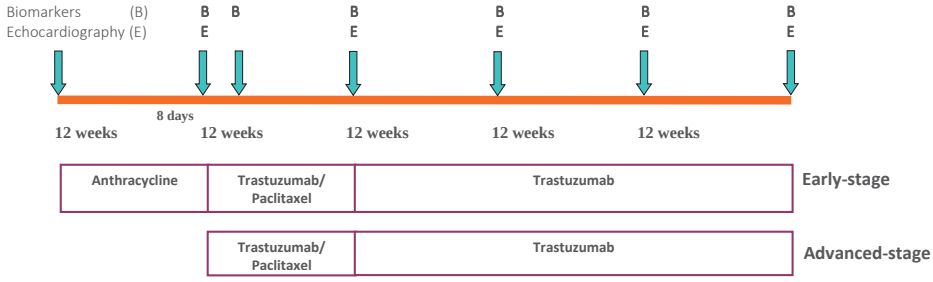


Figure S1. Study procedures during 1-year follow-up

Table S1. Overview of studies investigating the relation between NT-proBNP and cardiotoxicity

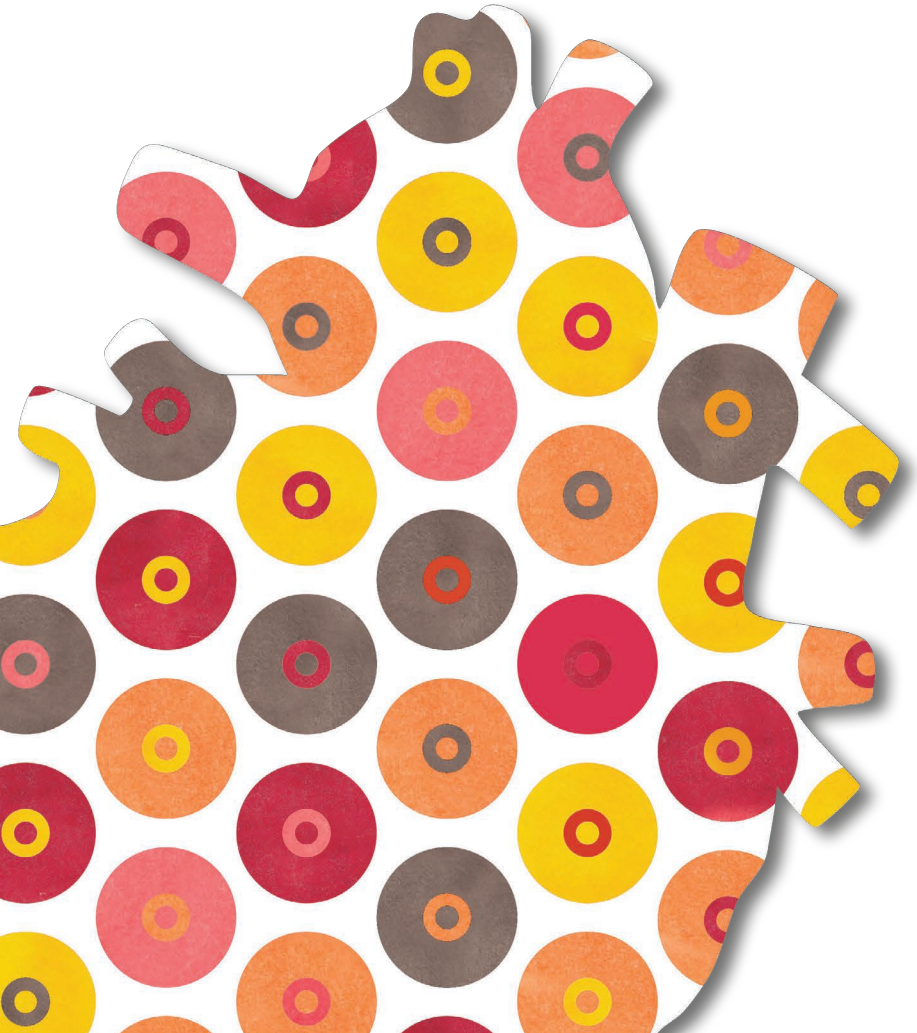
Study	Population	Treatment + duration	Sample size
Romano *et al.* (2011)	Breast cancer patients	AC	71
Bouwer *et al.* (2019)	HER2+ breast cancer patients	AC + T for 1 year	135
Zardavas *et al.* (2017)	HER2+ breast cancer patients	AC + T for 1 or 2 years	310
Putt *et al.* (2015)	HER2+ breast cancer patients	AC + T for 1 year	78
Sawaya *et al.* (2012)	HER2+ breast cancer patients	AC + T for 1 year	81
Fallah-rad *et al.* (2011)	HER2+ breast cancer patients	AC + T for 1 year	42
Ky *et al.* (2014)	HER2+ breast cancer patients	AC + T for 1 year	78
Ponde *et al.* (2017)	HER2+ breast cancer patients	Lap + Tax + T for 28 weeks	280
Sawaya *et al.* (2011)	HER2+ breast cancer patients	AC + T	43

Abbreviations: Ctox, cardiotoxicity; AC, anthracycline; T, trastuzumab; NT-proBNP, N-terminal pro-brain natriuretic peptide; LVEF, left ventricle ejection fraction; LVESV, left ventricle end-systolic volume; 2DE, two-dimensional echocardiography; NA, not available; HER2+, Human Epidermal growth factor Receptor 2 positive; 3DE, three-dimensional echocardiography.

Definition of ctox + imaging modality	Incidence of ctox	NT-proBNP changes during T	NT-proBNP related to ctox	NT-proBNP assays
LVEF of ≥20% and/or increase in LVESV of ≥15% from baseline measured with 2DE	NA	+	+	Elecsys
Absolute LVEF decline >10% from baseline and/or LVEF <45% measured with 3DE	33%	+	-	Dimension Vista 500
Asymptomatic absolute LVEF decline >10% from baseline and LVEF <50%	14%	+	-	Elecsys
Symptomatic absolute LVEF decline ≥5% to <55% or asymptomatic absolute LVEF decline ≥ 10% to <55% measured with 2DE	29%	-	-	Dimension Vista 500
Symptomatic absolute LVEF decline ≥5% to <55% or asymptomatic absolute LVEF decline ≥ 10% to <55% measured with 2DE	32%	-	-	Dimension Vista 500
LVEF decline >10% to <55% from baseline measured with 2DE with symptoms of heart failure	24%	-	-	Elecsys
Symptomatic absolute LVEF decline ≥5% to <55% or asymptomatic absolute LVEF decline ≥ 10% to <55% measured with 2DE	24%	-	-	Dimension Vista 500
Symptomatic congestive heart failure NYHA III or IV measured with 2DE	4%	-	-	Cobas®
Symptomatic LVEF decline of ≥5% to <55% or an asymptomatic LVEF decline ≥10% to <55% measured with 2DE	21%	-	-	Dimension Vista 500

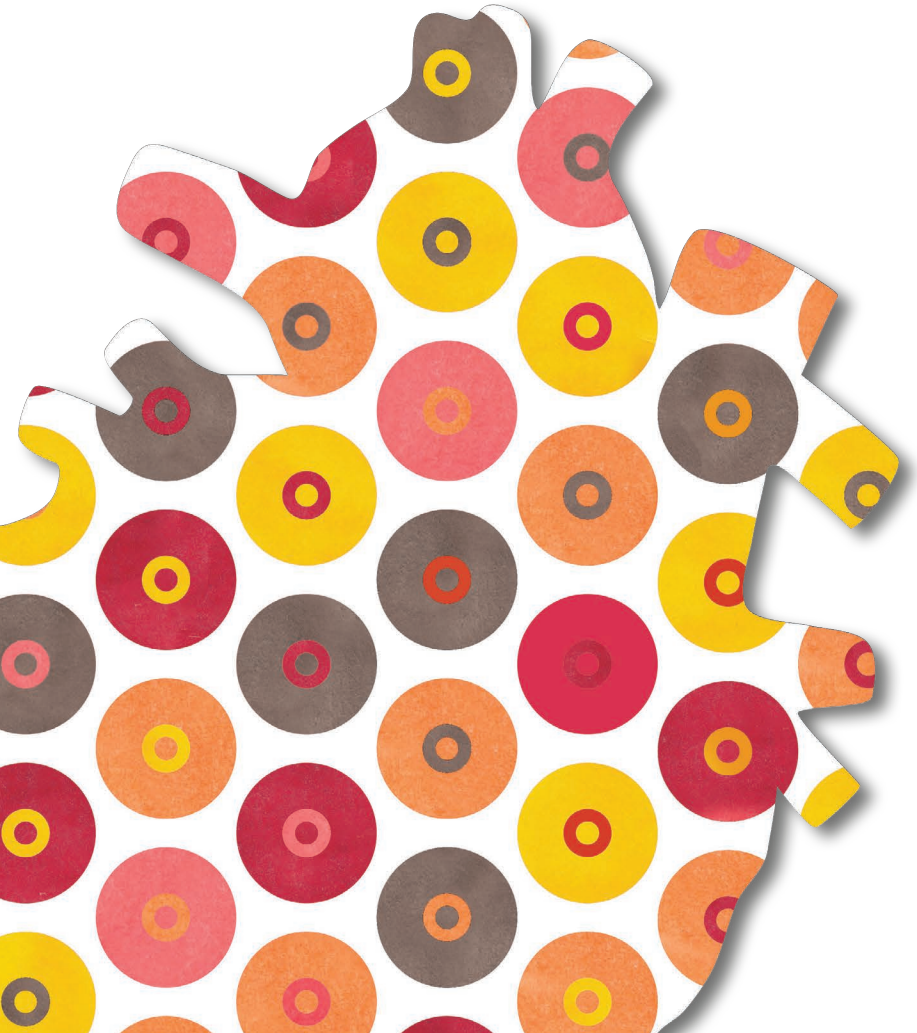
PART III

REVERSE CARDIO-ONCOLOGY: CANCER DEVELOPMENT IN PATIENTS WITH ARTERIAL THROMBOSIS



PART IV

EPILOGUE



EPILOGUE

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION AND CONCLUSIONS

This thesis aimed to contribute to the current body of evidence of anti-cancer therapy related cardiotoxicity, with particular focus on trastuzumab-induced cardiotoxicity during HER2-positive metastatic breast cancer (MBC). In addition, cancer development in patients with arterial thrombosis was studied for a better understanding of reverse cardio-oncology. In this chapter, the main findings that are described in this thesis are summarized and placed into a broader clinical context.

1. Incidence of trastuzumab-induced cardiotoxicity in patients with HER2-positive MBC
In **chapter 2**, we studied the incidence of cardiotoxicity in relation to trastuzumab treatment in 429 patients with HER2-positive MBC and a baseline (i.e. before trastuzumab treatment start) left ventricular ejection fraction (LVEF) ≥50%. These patients, who had a median age of 53 years, were treated in 8 different Dutch hospitals between 2000-2014. Cardiotoxicity, defined as a LVEF <50% at any moment during trastuzumab treatment, or a decline >10%-points from baseline, was as high as 11.7% after 1 year of trastuzumab treatment, and gradually decreased over time to a yearly risk of 7.1% after 4 years. The incidence of severe cardiotoxicity, defined as a LVEF <40% at any moment during follow-up, was estimated at 2.8% after 1 year, and remained at this low level thereafter. Factors that were associated with increased incidence of trastuzumab-induced cardiotoxicity included a baseline LVEF <60% (adjusted hazard ratio [aHR] 1.52), previous cardiotoxicity during neoadjuvant or adjuvant treatment with trastuzumab and/or anthracycline (aHR 4.48), and current smoking (aHR 1.73). The cumulative incidence of severe cardiotoxicity was limited to 3.1% after 4 years trastuzumab treatment in patients without any of these risk factors. Trastuzumab treatment was continued in 62% of patients with non-severe cardiotoxicity (LVEF <50%, but ≥40%), and in 24% of patients with severe cardiotoxicity. Interestingly, non-severe cardiotoxicity appeared reversible, defined as any LVEF increase to a value <5% below baseline, in 56% of patients in whom trastuzumab treatment was continued and in 57% of patients in whom trastuzumab treatment was permanently discontinued. In addition, severe cardiotoxicity appeared reversible in 33% of patients in whom trastuzumab treatment was continued and in 40% of patients in whom trastuzumab treatment was permanently discontinued.

In **chapter 3**, we studied the incidence of cardiotoxicity in patients with reduced cardiac function (baseline LVEF between 40 and 50%) treated with trastuzumab, a total of 37 patients with HER2-positive MBC. These patients, who had a median age of 52 years and median baseline LVEF of 46%, were treated with trastuzumab in eight different Dutch hospitals between 2000-2014, despite the fact that trastuzumab is contra-indicated in patients with a baseline LVEF below 50%.[1] The median trastuzumab treatment duration was 14 months

and the median overall survival (OS) was 47 months. During trastuzumab treatment, 35% of patients developed severe cardiotoxicity, defined as LVEF <40%. In addition, this severe cardiotoxicity was reversible in 54% of the patients and partly reversible, defined as any absolute LVEF increase ≥10%-points from nadir and to a value <5%-points below baseline, in 23% of patients. A total of 21 patients received cardio-protective medication (CPM), including beta-blockers and/or ACE-inhibitors, at the start or during trastuzumab treatment. Severe cardiotoxicity was reversible in 71% patients who received CPM during trastuzumab compared to 25% in patients without CPM from start trastuzumab. In addition, if patients received CPM after severe cardiotoxicity and discontinued trastuzumab, severe cardiotoxicity was reversible in 75%. After trastuzumab continuation in the same group, severe cardiotoxicity was reversible in 67% and partially reversible in 33% of patients.

In **chapter 4**, we studied radiological complete remission (rCR) and OS in 717 patients with HER2-positive MBC receiving first or second line trastuzumab-based therapy. These patients, who had a median age of 53 years, were also treated in eight different Dutch hospitals between 2000-2014. The 72 patients (10%) who achieved rCR demonstrated a median OS of 142 months, as compared to only 35 months in their counterparts with ongoing disease. Patients with oligo metastases (adjusted odds ratio [aOR] 4.19) and de novo MBC (aOR 5.14) were most likely to achieve rCR. Interestingly, patients who received trastuzumab as second-line treatment had lower odds of achieving rCR than those who received trastuzumab as a first-line treatment (aOR 0.26). In our cohort, trastuzumab was discontinued in 30 out of the 72 patients (43%) who achieved rCR. Of those 30 patients who discontinued trastuzumab, 22 patients (73%) remained disease-free for a median duration of 78 months.

2. Cardiac monitoring during trastuzumab treatment in patients with HER2-positive BC

In each individual patient with HER2-positive MBC, the risks and benefits of starting, continuing and discontinuing trastuzumab treatment need to be balanced to obtain the best cardiologic and oncological outcomes. Consequently, early detection of trastuzumab-induced cardiotoxicity is highly relevant. **Chapter 5** presents the state-of-the-art regarding cardiac monitoring strategies in patients with HER2-positive breast cancer receiving trastuzumab. The cardiac function of patients with HER2-positive breast cancer is most often monitored by periodically (i.e. every 3 months) measuring the LVEF with multigated acquisition (MUGA) scanning or two-dimensional echocardiography (2DE). A MUGA scan is a minimal invasive nuclear imaging technique that is widely available. However, the accuracy and reproducibility are questionable [2-4], whereas only limited information on cardiac structural dysfunction is obtained. Most importantly, a high cumulative radiation exposure of serial monitoring must be considered.[5] 2DE is another frequently used cardiac monitoring method, which is also non-invasive, highly

available and has low costs. However, 2DE is limited by a high inter-observer variability, dependency on image quality and insensitivity to detect small LVEF changes.[6, 7] Beside these two techniques, three-dimensional echocardiography (3DE) and cardiac magnetic resonance imaging (CMR) can be used to assess the LVEF. In fact, CMR is considered the gold standard for cardiac function and volume analyses. However, its high costs and (therefore) limited availability is the reason why CMR is not routinely used for serial LVEF monitoring. All in all, 3DE seems to be most suitable for cardiac monitoring because of its high reproducibility and accuracy, and its ability to assess the complete cardiac structure [8, 9], although operator experience is an important success factor.

Obviously, the use of imaging modalities is limited by their availability in clinical-oncological settings. Serological, cardiac biomarker based, monitoring strategies have potential advantages over imaging-based strategies, as these are less expensive, less time-consuming for patients and easier to perform. Serological strategies may even possibly detect cardiac damage at an earlier stage than certain imaging strategies. However, clinical biomarker studies investigating N-terminal fragment of the pro-hormone brain natriuretic peptide (NT-proBNP), troponin, C-reactive protein (CRP), myeloperoxidase (MPO), immunoglobulin E (IgE) and suppressor of tumorgenicity (ST2) showed conflicting results.

NT-proBNP is a well-known biomarker for the identification of heart failure.[10] Studies investigating the association between NT-proBNP and cardiotoxicity in patients with HER2-positive breast showed inconclusive results; 2 prospective studies observed increased NT-proBNP levels in patients with cardiotoxicity [11, 12], but in several other studies this was not observed.[13-17]

Troponin regulates the contractile element actin and myosin in the cardiomyocyte. Some studies in patients with HER2-positive breast cancer treated with anthracycline and trastuzumab showed a relationship between increased troponin values during trastuzumab treatment and an LVEF decline.[14, 18] However, various other studies in patients treated only with trastuzumab did not demonstrate this.[13, 15, 16, 19-21] These conflicting results might be explained by the timing of conventional troponin measurement and the preceding treatment with anthracycline.[22, 23] In addition, it is plausible that high-sensitive troponin assays have a better range for detection of trastuzumab-induced cardiotoxicity than conventional assays.

CRP is a marker of inflammation, which has prognostic value in patients with chronic heart failure.[24] A small study of 54 patients with HER2-positive breast cancer treated with trastuzumab showed that normal hs-CRP values are associated with a low risk of developing a LVEF decline.[21] However, other studies could not demonstrate this.[13, 16, 20]

MPO is a pro-inflammatory enzyme that is involved in the release of oxidative stress.[25] Two studies found that increases in MPO were associated with cardiotoxicity during trastuzumab treatment in patients with HER2-positive breast cancer.[14, 16]

IgE is involved in the immune system which has a role in maintaining myocardial homeostasis in patients with heart failure.[26] A small study of 7 patients with HER2-positive breast cancer treated with anthracycline and trastuzumab showed that high baseline IgE levels were associated with a low risk of cardiotoxicity.[27]

ST2 concentrations reflect cardiovascular stress and myocardial fibrosis.[28] Only 1 study has investigated the association between ST2 and cardiotoxicity, and showed that in 81 patients with HER2-positive breast cancer ST2 levels did not change during treatment, nor predicted subsequent development of cardiotoxicity.[17]

As stated before, the use of 2DE as cardiac monitoring technique has multiple limitations. However, the performance of 2DE in clinical oncology can potentially be improved by adding speckle tracking (ST). 2DE-ST is a sensitive imaging modality that provides opportunities for detecting subclinical cardiac dysfunction in patients receiving cancer therapy. In **chapter 6**, we studied the correlation between strain measurements based on 2DE-ST and CMR strain in 47 patients with HER2-positive breast cancer (81% with early-stage breast cancer (EBC) and 19% with MBC) during trastuzumab treatment. Patients were enrolled in the Albert Schweitzer Hospital, Dordrecht with a median age of 57 years and a median baseline LVEF of 66% measured with 2DE. Global longitudinal strain (GLS; Pearson's r=0.33) and global radial strain (GRS; r=0.09) based on 2DE-ST and CMR showed only weak correlations. Nevertheless, the change in 2DE-ST-measured GLS after 3 months trastuzumab was predictive of the change in CMR-measured LVEF after 6 months trastuzumab (p=0.021), whereas the change in 2DE-measured LVEF was not (p=0.292). Importantly, the 11 patients who developed cardiotoxicity, defined as LVEF <45% at any moment during trastuzumab treatment and/or LVEF decline >10%-points from baseline, had a larger 2DE-ST-measured GLS change after 3 months trastuzumab (+5.2%-points) than those who did not develop cardiotoxicity (+1.7%-points, p=0.016). Based on these findings, we conclude that speckle tracking might improve the performance of 2DE to predict LVEF changes in patients with HER2-positive breast cancer during trastuzumab treatment.

As described above, NT-proBNP is an important biomarker for left ventricular failure and a monitoring strategy based on serial NT-proBNP measurements has potency to (early) recognize trastuzumab-induced cardiotoxicity.[10] In **chapter 7**, we assessed the temporal patterns of NT-proBNP in 135 patients with HER2-positive breast cancer (84%

with EBC and 16% with MBC) during trastuzumab treatment, based on a median of 6 repeated measures of NT-proBNP per patient. Patients were enrolled in the Albert Schweitzer Hospital, Dordrecht, with a median age of 54 years and a median baseline LVEF of 62% measured with 3DE. During a maximal follow-up of 1 year (median of 6 months) a total of 45 patients (33%) developed trastuzumab-induced cardiotoxicity, defined as LVEF <45% at any moment during treatment and/or LVEF decline >10%-points from baseline, based on 3DE. Baseline NT-proBNP values were largely in normal range in the patients who developed cardiotoxicity (median value 12 pmol/l) as well in those in whom left ventricular function was maintained (8 pmol/l). Still, higher baseline NT-proBNP levels were associated with the development of cardiotoxicity (aHR 1.04 per pmol/l). Additionally, patients who developed cardiotoxicity showed a steeper increase in NT-proBNP (+10.2 pmol/l versus +2.5 pmol/l) and had on average higher NT-proBNP levels (16.8 pmol/l versus 10.1 pmol/l) during trastuzumab treatment than patients who did not develop cardiotoxicity. However, no steadily or sudden increase in NT-proBNP was observed prior to the development of cardiotoxicity.

3. Cancer development in patients with arterial thrombosis

In the third part of this thesis, the risk of cancer diagnosis after an acute ST-segment elevation myocardial infarction (STEMI) was investigated for a better understanding of the reverse cardio-oncology phenomenon, as multiple observational studies have demonstrated an increased risk of cancer after developing a STEMI. [29, 30] In **chapter 7**, we investigated 1.809 patients who were hospitalized for a STEMI and 10.052 participants of a large population-based cohort study, The Rotterdam Study. During total follow-up, there was no significant difference in the incidence of cancer diagnosis between STEMI patients and the general population (HR 0.96, 95%CI 0.78-1.19). However, in the first 3 months after STEMI, the hazard of cancer diagnosis was markedly higher compared to the general population (HR 2.45, 95%CI 1.13-5.30). Interestingly, among patients who developed STEMI higher CRP, higher platelets count, and lower hemoglobulin levels were significantly associated with increased risk of cancer diagnosis during the first year after STEMI (HRs 2.93 for CRP >10 mg/dL, 2.10 for platelet count >300*10^9, and 3.92 for hemoglobin <12 g/dL).

Conclusions

1. Serial cardiac monitoring is warranted in patients with HER2-positive MBC and certain risk factors

Exploring the incidence and reversibility of cardiotoxicity as result of trastuzumab treatment for HER2-positive MBC has led to a tailored cardiac monitoring recommendation. Cardiac monitoring could be tailored in order to not miss the patients who are at high risk for developing cardiotoxicity, but not over-treat those patients

who are at low risk. In this respect, our investigation provided important stratifying risk factors and showed that not every LVEF decline was reversible, which supports the use of cardiac monitoring in patients with HER2-positive MBC. Based on the findings of chapter 2, serial cardiac monitoring every 3 months is warranted during trastuzumab treatment of HER2-positive MBC patients with ≥1 of the found risk factors. However, less intensive monitoring can be in place in patients without risk factors.

2. A baseline LVEF <50% might not be an absolute contra-indication for trastuzumab treatment

Furthermore, we found a higher incidence of severe cardiotoxicity in patients with HER2-positive MBC who start trastuzumab despite their reduced LVEF of <50% compared to those with LVEF ≥50%. However, cardiotoxicity was reversible in most patients with LVEF <50% at baseline, which might be explained by the fact that some patients received CPM. Interestingly, the OS in patients with HER2-positive MBC who received trastuzumab with a baseline LVEF <50% was comparable with those who had a baseline LVEF ≥50%. These findings suggests that a baseline LVEF <50% might not be an absolute contra-indication for receiving trastuzumab if optimized cardiac management is provided. Potentially, more patients with HER2-positive MBC could benefit from trastuzumab treatment. However, for now, additional research is necessary before patients with a baseline LVEF <50% can be safely treated with trastuzumab.

3. Discontinuation of trastuzumab after rCR should be carefully considered

Safe de-escalation of trastuzumab treatment in patients with HER2-positive MBC who achieved rCR is important to reduce cardiotoxicity. Two thirds of patients who discontinue trastuzumab because of rCR remained in complete remission for several years. Unfortunately, whether it is safe to stop trastuzumab in all patients who develop rCR could not be demonstrated. Decisions to discontinue trastuzumab after achieving rCR should be carefully considered between physicians and patients, until further research has been performed.

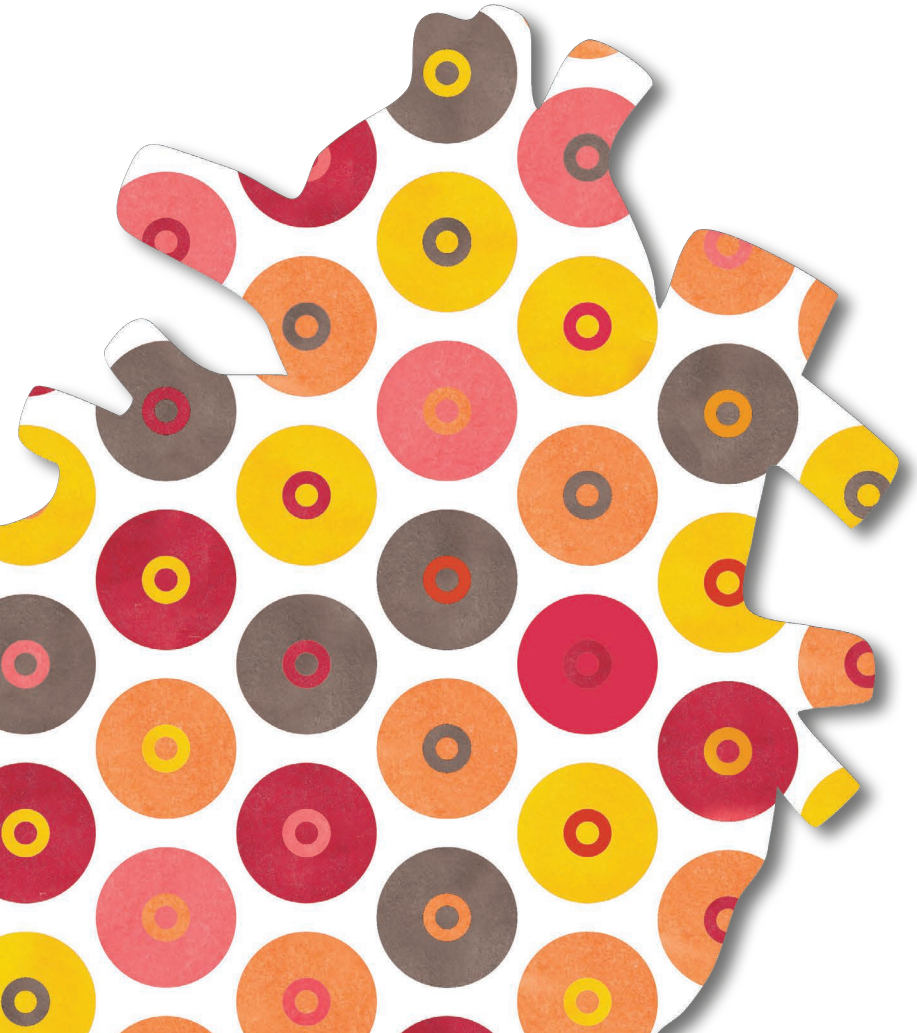
4. GLS measurement with 2DE-ST could predict cardiotoxicity earlier and with greater accuracy

Current cardiac monitoring of patients with HER2-positive breast cancer treated with trastuzumab primarily consists of MUGA scan, or 2DE with or without ST. However, 3DE seems to be most suitable for cardiac monitoring during trastuzumab treatment for HER2-positive breast cancer. In addition, we showed that with 2DE-ST cardio-toxic changes could be predicted with greater accuracy, although there is room for further improvement.[31, 32] An systematic review, including 1504 patients, demonstrated that an early relative reduction in GLS of 10% to 15%-points during treatment is very likely to be

indicative for cardiotoxicity.[32] In addition, a recent meta-analysis showed that also the absolute GLS cut-off values, ranging from -21% to -14% can be useful to stratify patients at high risk of developing cardiotoxicity, which is especially important in patients without baseline cardiac imaging.[31]

5. Further research is warranted on the role of NT-proBNP to identify cardiac dysfunction
Patients who developed trastuzumab-induced cardiotoxicity had on average higher NT-proBNP levels and steeper increases in NT-proBNP than patients without cardiotoxicity, but the changes were too subtle and have limited clinically relevance. [33] Based on these results, we concluded that NT-proBNP is currently not suitable to be used as a biomarker for early identification of trastuzumab-induced cardiotoxicity. Recently, the largest (n=323) study with the longest follow-up (maximum of 3.7 years) up until this date showed that doubling of NT-proBNP levels resulted in lower LVEFs (-1.1%) and a higher probability of developing cardiotoxicity (HR 1.18).[34] Additionally, they found that the use of certain thresholds, i.e. of 150 ng/L or 300 ng/L, may be useful for identifying subgroups at increased risk for cardiotoxicity with a high specificity and positive predictive value. These results show that the debate regarding the use of NT-proBNP, alone or in combination with other biomarkers, for identification of cardiac dysfunction, especially in patients treated with anthracycline and trastuzumab, is not yet over.

6. STEMI patients (with certain risk factors) have increased risks of cancer diagnosis
STEMI might be a paraneoplastic manifestation of yet to be diagnose cancer as during the initial months after hospitalization, STEMI patients appeared at increased risk of being diagnosed with cancer. This can be explained by the fact that some STEMIs are of paraneoplastic origin, for instance due to a pro-thrombotic state. However, residual confounding through shared risk factors cannot be ruled out. STEMI as a paraneoplastic manifestation of diagnosed cancer is hallmarked by a pro-inflammatory status and anemia as patients with higher CRP and lower haemoglobin levels after STEMI have increased risks of cancer diagnosis. Although the etiology of the association of arterial thrombotic occlusions (STEMI) and cancer diagnosis is not yet known, physicians who treat STEMI patients with ≥1 of the before mentioned risk factors should be aware of the increased risk of cancer.



EPILOGUE

CLINICAL PERSPECTIVES AND FUTURE DIRECTIONS

CLINICAL PERSPECTIVES AND FUTURE DIRECTIONS

Ongoing research in patients with breast cancer over the past decennia has improved breast cancer-related survival due to earlier detection and better therapeutic approaches. This along with an aging population and interlinkage of cancer and cardiovascular diseases through common risk factors, including obesity, diabetes, hypertension and current smoking [35], has led to an extensive growth in the incidence of anti-cancer treatment-induced cardiotoxicities. Recognizing those patients who are at increased risk for treatment-induced cardiotoxicity is an unsolved clinical challenge. The investigation of cardiac dysfunction in patients with HER2-positive breast cancer treated with trastuzumab and anthracycline, presented in this thesis, may help in this respect.

Baseline risk assessment

Ultimately and ideally, cardiovascular risk assessment should be performed prior to starting potentially cardio-toxic cancer treatment, including anthracycline and HER2-targeted therapies such as trastuzumab.[36] For patients with HER2-positive breast cancer, application of risk stratification before the start of treatment will improve personalized approaches to minimise the risk of cardiotoxicity as result of trastuzumab and/or anthracycline treatment. A strategy of routine cardiac monitoring during trastuzumab treatment in combination with early implementation of cardio-protective medications may decrease cardiotoxicity and the risk of trastuzumab interruption.[37-39] Additionally, several guidelines and expert position papers have recommended baseline cardiovascular risk assessment in cancer patients prior to starting possible cardio-toxic treatment.[2, 36, 40-42]

In **chapter 2**, we provided stratifying baseline risk factors for cardiotoxicity during (long-term) trastuzumab treatment in patients with HER2-positive MBC. Importantly, all risk assessments contain a variety of risk factors including medical cardiovascular risk factors, lifestyle cardiovascular risk factors and type of cardio-toxic cancer treatment. However, up until now, there is no universal and standardized risk assessment to facilitate cardiovascular risk stratification. In addition, current risk factors, including those found in **chapter 2**, have wide confidence intervals making it difficult to clearly distinguish between those patients who develop cardiotoxicity and those in whom the left ventricular function remains preserved. Therefore, a prospective risk stratification tool could be developed combining known or new risk factors, cardiac monitoring parameters, serologically measured biomarkers and genetic factors to provide more specific risk predictions for individual patients. Ideally, this tool should be externally validated in large datasets in order to investigate the generalizability and transportability in another population.

Cardiac monitoring

Important questions regarding cardiac monitoring of patients with HER2-positive breast cancer treated with trastuzumab are: in whom, how, for how long and how often should cardiac monitoring be performed? Ideally, monitoring of the LVEF is performed with CMR as this technique is considered the gold standard for cardiac function and volume analyses. However, the high costs and therefore limited availability are important reasons why CMR is currently not used and will not be used for cardiac function monitoring before and during trastuzumab treatment.

In whom is cardiac monitoring necessary?

Baseline risk assessment before trastuzumab initiation is crucial to stratify cancer patients into low or high risk for cardiotoxicity on which consequently the frequency of cardiac monitoring is based. Common clinical factors that may indicate that a patient is at high risk for developing cardiac dysfunction during anti-cancer treatment are baseline LVEF <50%, prior anthracycline-based treatment, age >75 years or <10 years, prior mediastinal or chest radiotherapy, hypertension, previous combined treated with anthracycline and trastuzumab, and diabetes mellitus.[41] In **chapter 3,** we found that despite having a LVEF <50% before trastuzumab initiation, more than halve of patients with HER2-positive MBC did not develop severe cardiotoxicity, defined as LVEF <40% during trastuzumab treatment. Additionally, omitting anthracycline might be an attractive approach for the treatment of stage II – III HER2-positive breast cancer due to the decreased toxicity and limited rCR benefit.[43, 44] This could result in lower rates of cardiotoxicity and therefore a less strict cardiac monitoring strategy may be in place and resources could be focussed on the patients with HER2-positive breast cancer at highest risk for cardiotoxicity. Specifically, for patients with HER2-positive MBC treated with trastuzumab we found in **chapter 2** that cardiac monitoring is recommended in cases of ≥1 of the following risk factors: LVEF <60%, current smoking or cardiotoxicity during prior neoadjuvant or adjuvant treatment with trastuzumab and/or anthracycline. This also implies that cardiac monitoring could be omitted in patients without these risk factors due to the low incidence of severe cardiotoxicity in this group.[45] For patients with HER2-positive EBC, additional discriminating risk factors are needed and could be found in larger, prospective studies.

How should cardiac monitoring be performed?

For now, cardiac monitoring should preferably be performed with 3DE as this is a reproducible, accurate and non-irradiating imaging technique.[2, 41] Additionally, 3DE has lower intra and inter-observer variability, and temporal variability when compared with 2DE.[7, 46] Nonetheless, it should be mentioned that the applicability of 3DE is

also still limited in the oncologic setting due to limited availability in oncological centers and requirement of adequate image quality and operator experience for 3DE. Importantly, for each patient, the same imaging technique at the same center is recommended for serial follow-up.

In addition to LVEF monitoring, GLS measurement with 2DE-ST may be considered. This because with 2DE-ST, cardio-toxic changes could be predicted with greater accuracy and earlier, as an GLS decline precedes an LVEF decline.[17, 31, 32, 47-49] In **chapter 6**, we found that addition of ST improved the performance of 2DE to predict cardio-toxic changes. However, it should be mentioned that widely application of ST in clinical practice is limited by the need for high-resolution image quality, operator experience and the insufficient standardization of measurements by different echocardiographic vendors resulting in non-comparative results.[50] A great effort has been made to overcome these limitations and to standardize the measurements of different vendors and to automatize GLS calculation in order to offer immediate results to the clinicians without errors in the calculations.

Additionally, measuring cardiac biomarkers may also be a non-invasively diagnostic tool for identification of cardiotoxicity. Hypothetically, measuring a combination of promising biomarkers such as high sensitive troponin, NT-proBNP (which was investigated in **chapter 7**) and MPO could increase the predictive ability. Future research is required to determine the optimal (combination of) biomarker(s), timing of biomarker analysis, cut-off values for cardiotoxicity and the optimal intervention required based on these biomarkers results.

For how long should cardiac monitoring be performed?
In HER2-positive EBC, for which the maximum duration of trastuzumab is 1 year, it is recommended to perform serial cardiac monitoring every 3 months up until trastuzumab completion.[40, 50] The additional value of serial cardiac monitoring after discontinuation of trastuzumab treatment has not been confirmed yet.[51] Based on current knowledge, it would be reasonable to consider omission of cardiac monitoring after discontinuation of trastuzumab treatment. In patients with HER2-positive MBC, in which trastuzumab can be continued beyond disease progression,[52] investigated in **chapter 2** we found that the additional value of cardiac monitoring in patients with ≥1 of the before mentioned risk factors is low after 3 years trastuzumab, as the yearly increase and absolute numbers of cardiotoxicity were low after this period.[45]

How often should cardiac monitoring be applied?
The optimal frequency of cardiac monitoring during trastuzumab treatment has not been established yet. After the approval of trastuzumab in 2006, the U.S. Food and Drug Administration (FDA) recommended for patients with asymptomatic HER2-positive EBC

undergoing adjuvant trastuzumab treatment serial cardiac surveillance every 3 months, which has been adopted in numerous guidelines.[50, 53]. However, the American Society of Clinical Oncology (ASCO) and European Society of Medical Oncology (ESMO) recommend an individualized cardiac surveillance scheme during cardio-toxic anti-cancer treatment based on the patients' risk rather than a one-size-fits all approach.[40, 41] In **chapter 5**, we showed that on average cardiac monitoring is performed every 3 months during trastuzumab treatment for HER2-positive EBC and more infrequently during trastuzumab treatment for HER2-positive MBC.[51] More and larger prospective studies are urgently awaited to investigate the optimal frequency of cardiac monitoring and whether a personalized cardiac monitoring scheme based on risk factors might be beneficial.

Interventions for prevention and treatment of cardiotoxicity

Cardio-protective medications could both prevent and treat trastuzumab-induced cardiotoxicity, as it has been shown that cardio-protective medications have a (modest) positive effect on the cardiac function.[54-56] A meta-analysis including 2302 patients showed that cardio-protective medications including angiotensin-converting-enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARB) and beta-blockers reduced the incidence of heart failure and had better LVEF preservation in patients who received anthracycline treatment, however, not in those who simultaneously received trastuzumab.[54] Currently, treatment of trastuzumab-induced cardiotoxicity consists of ACE-inhibitors (or ARB) and/or beta-blockers which is based on the current treatment of heart failure.[50, 57] Further research is needed to optimize the combination of cardio-protective therapies to reduce trastuzumab-induced cardiotoxicity and to identify populations with the greatest benefit of treatment.

Moreover, trastuzumab-induced cardiotoxicity could eventually lead to clinically manifest heart failure, which has a worse prognosis than heart failure due to any other causes, as for instance due to ischemic heart disease.[58] Recent developments in heart failure medications such as angiotensin receptor neprilysin inhibitors (ARNIs) and sodium-glucose cotransporters-2 (SGLT2) inhibitors has demonstrated some positive outcomes in chronic heart failure patients.[59] However, the additional value of these cardio-protective medications in trastuzumab-induced cardiotoxicity remains to be investigated. In patients with end-stage heart failure, heart transplantation is the gold standard therapy option.[60] However, the ineligibility of oncological patients and shortage of donors has limited the possibility of heart transplantations in patients with HER2-positive breast cancer. Other frequently used treatment options for patients with end-stage heart failure are implantable cardioverter defibrillators (ICDs) to prevent arrhythmic sudden cardiac death or continuous-flow left ventricular assisted device (LVAD) therapy as a bridge to transplantation therapy.[57, 61]

On the other hand, trastuzumab-induced cardiotoxicity can also be decreased by interruption of trastuzumab, which is not desirable due to a poor oncological prognosis.[62, 63] In **chapter 4**, we showed that in case of development of rCR, two thirds of patients who discontinued trastuzumab for HER2-positive MBC remained in complete remission for several years. Unfortunately, we could not conclude whether it is safe to stop trastuzumab in all patients who develop a rCR. Importantly, physicians should balance the cardiovascular risks and oncological benefits of trastuzumab treatment in every patient, and this balance may differ between patients with HER2-positive EBC or HER2-positive MBC.

Cardio-Oncology care

This all highlights the importance of early cardiovascular risk factor assessment and modification, routine cardiac monitoring and early implementation of cardio-protective medications, if necessary. This can be provided by a multidisciplinary collaboration between oncologists, hematologists, radiotherapists, cardiologists and allied health care professionals in a so-called Cardio-Oncology service. The scope of Cardio-Oncology care is not only aimed at prevention, detection, monitoring and treatment of cardiotoxicity related to anti-cancer treatment, but also at providing best practice, guidelines-directed cancer care by increasing the proportion of cancer patients who complete their treatment without interruption, and at the development of future novel anti-cancer treatments that have less or minimal impact on the cardiovascular health of the cancer patient. The number of new cancer treatments has grown exponentially over the last decennium and many can result in varying degrees of cardiotoxicity.[64] Close collaboration in a Cardio-Oncology service will help to ensure optimal care for cancer patients without compromising cardiovascular health. A retrospective study in patients receiving anthracycline treatment for a variety of cancer types showed that patients with cardiac consultation, preferably by a cardio-oncology specialist, received more cardio-protective medications and had lower all-cause mortality, compared to patients without cardiac consultation.[65] In addition, another retrospective study showed that cardiac consultation occurred in 28% of patients receiving trastuzumab for HER2-positive breast cancer which was associated with higher adherence to guideline of cardiac monitoring, an lower systolic blood pressure and a lower BMI in patients with baseline BMI ≥25 kg/m^2.[66] Furthermore, targeted cardiac consultation in patients who develop cardiotoxicity during trastuzumab treatment, resulted in completion of trastuzumab treatment with higher LVEFs (52% versus 48%) and better overall survival (98 months versus 78 months) compared to patients who did not receive targeted cardiac consultation.[67]

Furthermore, collaboration in Cardio-Oncology service can also be used to build a large, prospective, national consortium such as the Borstkanker Onderzoek Groep (BOOG) or Hemato-Oncologie Stichting Volwassenen Nederland (HOVON) with the ultimate goal of

establishing optimal strategies for cardio-oncological care. Recently, in the Netherlands, the ONCOR registry, which is a national, multicentre, observational cohort study, has been set up at different hospitals with a standardised cardio-oncology care pathway.[42, 68] The goal of the ONCOR registry is two-fold, namely prospective data collection and evaluation of cardio-oncology care in daily practice. The next step forward in this consortium would be the incorporation of a prospectively validated risk stratification tool for every targeted anti-cancer therapy to accurately discriminate patients into high and low risk for cardiotoxicity. Furthermore, addition of a biobank to the current registry can contribute to the identification of potential biomarkers and genetic factors, which may eventually will enhance etiologic, diagnostic and prognostic knowledge of anti-cancer treatment-induced cardiotoxicity. Lastly, the registry can also be used as a pragmatic trial to investigate new interventional therapeutic strategies and to determine the optimal cardiac monitoring strategy for each cardiotoxic anti-cancer therapy.

Concluding, in patients with HER2-positive breast cancer, the cardiovascular risks of trastuzumab treatment must be weighed individually and carefully against the improved oncological prognosis as result of trastuzumab treatment. Cardio-Oncology services, that stimulate close collaboration between the involved specialists, can help in this respect and have shown to have a beneficial effect on both the cardiovascular and oncological outcome. However, currently, not every center has access to a Cardio-Oncology service or a dedicated cardio-oncology team with the possibility to monitor and address cardiotoxicity in patients with HER2-positve breast cancer. Increased prospective research, for instance allied in a consortium, can help to establish optimal monitoring and treatment strategies for the cardio-oncological care.

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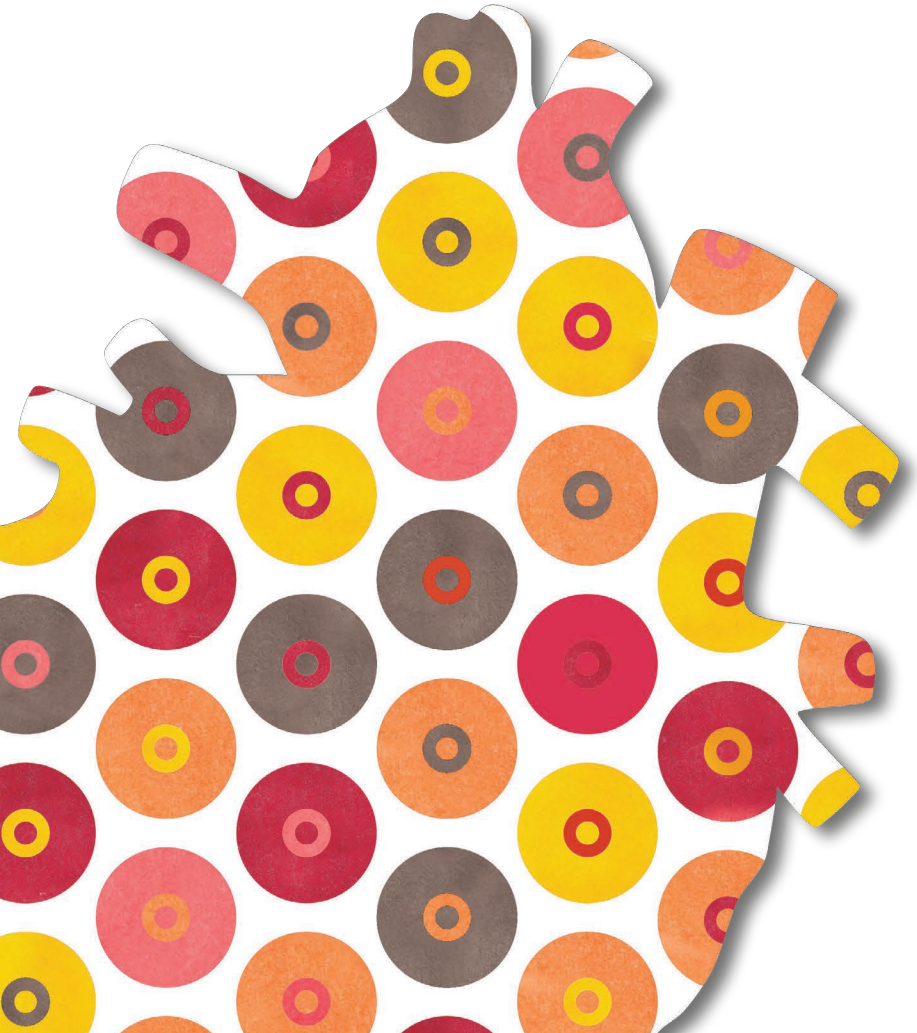

EPILOGUE

Dutch summary -
Nederlandse samenvatting

DUTCH SUMMARY - NEDERLANDSE SAMENVATTING

Het onderwerp van dit proefschrift is anti-kanker behandeling gerelateerde cardiotoxiciteit, en in het bijzonder cardiotoxiciteit als gevolg van trastuzumab behandeling in patiënten met HER2-positieve borstkanker. Het herkennen van patiënten met een verhoogd risico voor anti-kanker behandeling geïnduceerde cardiotoxiciteit is een belangrijk klinisch probleem waar tot op heden nog geen goede oplossing voor is gevonden. Het belangrijkste doel van dit proefschrift was om een bijdrage te leveren aan de huidige literatuur en kennis met betrekking tot het monitoren en herkennen van trastuzumab geïnduceerde cardiotoxiciteit in patiënten met HER2-positieve borstkanker. Daarnaast hebben we de ontwikkeling van kanker bestudeerd in patiënten met arteriële trombose om zo een beter begrip te krijgen over het omgekeerde cardio-oncologie fenomeen.

1. Incidentie van trastuzumab geïnduceerde cardiotoxiciteit in patiënten met HER2-positieve gemetastaseerde borstkanker

Aangezien de mediane overall survival (OS) van patiënten met HER2-positieve gemetastaseerde borstkanker inmiddels meer dan 4 jaar is met continu trastuzumab gebruik, moeten de voordelen, zoals verbeterde overleving, en de nadelen, zoals met name cardiotoxiciteit, van het starten, doorgaan en stoppen van trastuzumab behandeling zorgvuldig worden overwogen om zo de beste uitkomst voor de patiënten te behalen.[1, 2] Hierbij is gerichte cardiale monitoring van belang om patiënten met een verhoogd risico op cardiotoxiciteit niet te missen, maar om patiënten met een laag risico niet over te behandelen. In **hoofdstuk 2** onderzochten we daarom de incidentie en reversibiliteit van trastuzumab geïnduceerde cardiotoxiciteit om zo een gericht advies te kunnen geven over de cardiale monitoring in patiënten met HER2-positieve borstkanker. In deze studie zagen we dat niet elke daling van de linkerventrikel ejectiefractie (LVEF) reversibel was, wat het belang van cardiale monitoring strategieën benadrukt. Daarnaast was de incidentie van cardiotoxiciteit, gedefinieerd als een LVEF <50% of daling van meer dan 10%-punten vanaf de baseline waarde, en ernstige cardiotoxiciteit, gedefinieerd als een LVEF <40%, in het eerste jaar van trastuzumab behandeling het hoogst (11.7%) welke daalden de jaren daaropvolgend. Factoren die geassocieerd waren met het ontwikkelen van cardiotoxiciteit waren een baseline LVEF <60% (adjusted hazard ratio [aHR] 1.52), eerdere cardiotoxiciteit gedurende neoadjuvante of adjuvante behandeling met trastuzumab en/of anthracycline (aHR 4.48) en actief roken (aHR 1.73). Verder vonden we dat de cumulatieve incidentie van ernstige cardiotoxiciteit erg laag was, namelijk maar 3.1% na 4 jaar trastuzumab behandeling in patiënten zonder de eerdergenoemde relevante risicofactoren (LVEF op baseline van onder de 60%, eerdere cardiotoxiciteit gedurende neoadjuvante of adjuvante behandeling met trastuzumab en/of anthracycline en actief roken).

Vervolgens hebben we in **hoofdstuk 3** de incidentie van cardiotoxiciteit verder onderzocht in patiënten met HER2-positieve gemetastaseerde borstkanker die ondanks een verlaagde LVEF op baseline (baseline LVEF tussen de 40 - 50%) werden behandeld met trastuzumab. Ondanks dat de Food and Drug Administration (FDA) en European Medicines Agency (EMA) aanbeveelt om trastuzumab niet te starten in patiënten met een baseline LVEF onder de 50%, waren er 37 patiënten in onze multicenter cohort die trastuzumab hadden ontvangen met een mediane duur van 14 maanden.[3, 4] Deze patiënten hadden een mediane OS van 47 maanden, wat vergelijkbaar is met de OS van patiënten met HER2-positieve borstkanker die trastuzumab ontvangen met een baseline LVEF van 50% of meer (**hoofdstuk 2**). Bijna twee derde van de patiënten met een verlaagde baseline LVEF ontwikkelende geen ernstige cardiotoxiciteit gedefinieerd als een LVEF van onder de 40%. Daar komt nog eens bij dat wanneer patiënten ernstige cardiotoxiciteit ontwikkelden, dit in meer dan 2 van de 3 gevallen reversibel was, gedefinieerd als LVEF toename van ≥10%-punten tot een waarde van <5% onder de oorspronkelijke baseline LVEF-waarde. Verder zagen we een trend naar hogere percentages reversibiliteit in patiënten die cardiotoxiciteit ontwikkelden en behandeld werden met cardio-protectieve medicatie zoals ACE-remmers, beta-blokkers en angiotensine-2 inhibitoren.

Ten slotte hebben we in **hoofdstuk 4** de radiologische complete remissie (rCR) en OS bestudeerd in het gehele cohort van 717 patiënten met HER2-positieve gemetastaseerde borstkanker die zijn behandeld met trastuzumab in de eerste- of tweede lijn. Gedurende totale follow-up, bereikten 72 patiënten (10%) een rCR met een mediane OS van 142 maanden wat significant hoger was vergeleken met de OS van 35 maanden in patiënten met actieve ziekte. Verder hadden patiënten met oligo metastases (minder dan 3 metastasen) en de novo gemetastaseerde borstkanker een hogere kans op het bereiken een rCR (adjusted odds [aOR] ratio van 4.19 en 5.14). Interessant genoeg hadden patiënten die trastuzumab als tweedelijns behandeling kregen een lagere kans op het bereiken van een rCR dan patiënten die trastuzumab kregen als eerstelijnsbehandeling (aOR 0.26). Verder hebben we ook gekeken naar de-escalatie van trastuzumab in patiënten die rCR hadden bereikt, aangezien dit ook een belangrijke manier is om mogelijke cardiotoxiciteit te verminderen. In het gehele cohort werd trastuzumab gestopt in 30 van de 72 patiënten (43%) die een rCR hadden bereikt. Van deze 30 patiënten bleven er 22 patiënten (73%) ziekte vrij voor een mediane duur van 78 maanden. Op basis van deze resultaten in hoofdstuk 4 kunnen we niet concluderen of het veilig is om trastuzumab te stoppen in alle patiënten die een rCR bereiken.

2. Seriële cardiale monitoring in patiënten met HER2-positieve borstkanker

In **hoofdstuk 5** presenteerden wij een overzicht van de huidige literatuur over cardiale monitoring strategieën voor patiënten met HER2-positieve borstkanker die behandeld worden met trastuzumab. Over het algemeen wordt de cardiale functie (LVEF) van patiënten met HER2-positieve borstkanker periodiek, elke 3 maanden, met behulp van een multigated acquisition (MUGA) scan of 2-dimensionele echocardiografie (2DE) gemeten. Daarnaast kan de LVEF bepaald worden met een 3-dimensionele echocardiografie (3DE) en cardiale MRI (CMR). De CMR is de gouden standaard voor het bepalen van de cardiale functie en ventrikel volumina. Echter, door hoge kosten en daardoor beperkte beschikbaarheid van de CMR wordt de CMR momenteel niet gebruikt voor seriële metingen van de LVEF in patiënten met HER2-positieve borstkanker. Concluderend, lijkt 3DE de meest geschikte techniek voor cardiale monitoring van patiënten met HER2-positieve borstkanker gedurende trastuzumab behandeling vanwege een hoge reproducibiliteit en nauwkeurigheid, en aangezien het de complete cardiale structuur kan beoordelen.

Naast het meten van de LVEF, is het meten van strain parameters zoals de global longitudinal strain (GLS) en global radial strain (GRS) met 2D-speckle tracking echocardiografie (2DE-ST) ook een geschikte manier om (subklinische) cardiotoxiciteit op te sporen aangezien veranderingen in de strain vaak eerder en nauwkeuriger kunnen worden opgespoord dan veranderingen in de LVEF. In **hoofdstuk 6** hebben we de correlatie bestudeerd tussen strain metingen met de 2DE-ST en CMR, en met CMR-LVEF in 47 patiënten met HER2-positive borstkanker waarvan bij 19% sprake was van gemetastaseerde borstkanker. Alhoewel de correlatie tussen 2DE-ST en CMR matig was, vonden we in **hoofdstuk 6** wel dat een verandering in met 2DE-ST gemeten GLS na 3 maanden trastuzumab gerelateerd was aan een verandering in CMR gemeten LVEF na 6 maanden trastuzumab (p=0.021). Dit terwijl de verandering in met 2DE gemeten LVEF niet gerelateerd was aan een verandering CMR gemeten LVEF na 6 maanden trastuzumab (p=0.292). Verder vonden we dat in de 11 patiënten (28%) die cardiotoxiciteit ontwikkelden, gedefinieerd als LVEF <45% en/of daling >10%-punten van baseline, een grotere met 2DE-ST gemeten GLS verandering hadden (+5.2%-punten) vergeleken met patiënten die geen cardiotoxiciteit hadden ontwikkeld na 6 maanden trastuzumab (+1.7%-punten, p=0.016). Deze resultaten laten zien dat met het toevoegen van speckle tracking (ST) aan 2DE cardiotoxische veranderingen eerder voorspeld kunnen worden, aangezien een daling in de GLS daling voorafgaand gaat aan een daling in de LVEF. Belangrijk hierbij om te vermelden is dat de invoering van ST afhankelijk is van hoge resolutie kwaliteit echobeelden en ervaring van de echografist.

Daarnaast zijn cardiale biomarkers potentieel geschikt om te worden ingezet als cardiale monitoring techniek voor het detecteren van cardiotoxiciteit in patiënten met HER2-positieve borstkanker, aangezien het meten van biomarkers goedkoop is, minder tijdrovend, makkelijker en minder invasief voor de patiënt. In theorie, is het zelfs mogelijk dat biomarkers de cardiale schade in een eerder stadium kunnen detecteren dan bepaalde cardiale afbeeldingstechnieken. In **hoofdstuk 5** vonden we dat het meten van high sensitive troponine, N-terminal fragment van de pro-hormoon brain natriuretisch peptide (NT-proBNP) en myeloperoxidase (MPO) veelbelovend zijn voor het detecteren van cardiotoxiciteit aangezien NT-proBNP een biomarker is die verhoogd is bij hartfalen en MPO verhoogd is in het geval van oxidatieve stress.[5, 6] Meer onderzoek is nodig naar de optimale (combinatie van) biomarker(s), juiste meetmoment en afkapwaarde voor cardiotoxiciteit. In **hoofdstuk 7** onderzochten we verder of herhaalde metingen van NT-proBNP geschikt is voor het detecteren van cardiotoxiciteit in een cohort van 125 patiënten met HER2-positieve borstkanker gedurende trastuzumab behandeling. In totaal ontwikkelden 45 patiënten (33%) cardiotoxiciteit, gedefinieerd als LVEF <45% en/of daling >10%-punten van baseline, gedurende een maximale follow-up van 1 jaar (mediane follow-up van 6 maanden). In deze studie vonden we dat de baseline NT-proBNP waarde geassocieerd was met de ontwikkeling van cardiotoxiciteit gedurende trastuzumab behandeling met een aHR van 1.04 per 1 pmol/l toename in NT-proBNP. Patiënten die cardiotoxiciteit hadden ontwikkeld, hadden vervolgens hogere waardes van NT-proBNP (16.8 pmol/l versus 10.1 pmol/l) en lieten een snellere toename zien van NT-proBNP (+10.2 pmol/l versus +2.5 pmol/) vergeleken met patiënten die geen cardiotoxiciteit hadden ontwikkeld gedurende de follow-up. Ook zagen we geen plotselinge of gelijke toename in NT-proBNP waardes voor het ontwikkelen van cardiotoxiciteit.

3. Ontwikkeling van kanker in patiënten met arteriële trombose

Tot slot, onderzochten we in **hoofdstuk 7** het risico op het ontwikkelen van kanker na een acuut ST-segment elevatie myocard infarct (STEMI) met als doel om een beter begrip te krijgen over het omgekeerde cardio-oncologie fenomeen, aangezien meerder observationele studies hebben aangetoond dat er een verhoogd risico is op een kanker diagnose na het ontwikkelen van een STEMI.[7, 8] In deze studie onderzochten we 1809 patiënten die waren opgenomen in het ziekenhuis vanwege een STEMI en vergeleken we de kanker incidentie van deze groep met 10.052 deelnemers van de Rotterdam Study.[9] Gedurende de 5 jaar follow-up, werden er 115 STEMI patiënten en 677 deelnemers van de Rotterdam Study gediagnosticeerd met kanker (incidence rates 16.5 en 14.3 per 1000 person-years). Er werd geen verschil geobserveerd in de incidentie van kanker tussen de STEMI-patiënten en de algemene bevolking (HR 0.96, 95% CI 0.78-1.19). Desalniettemin, de hazard op kanker diagnose was significant hoger in de eerste 3 maanden na een STEMI vergeleken met de algemene bevolking (HR 3.20, 95%

CI 1.65 – 6.19). Verder vonden we dat STEMI-patiënten met hogere CRP-waarde (HR 2.93 voor CRP >10 mg/dL), hogere trombocyten waarde (HR 2.10 voor trombocyten >300*10^9/L) en lagere hemoglobine waarde (HR 3.92 voor hemoglobine <12 g/dL) een verhoogd risico hebben op kanker diagnose in het eerste jaar na het ontwikkelen van een STEMI.

Conclusies

1. Seriële cardiale monitoring is van belang in patiënten met HER2-positieve gemetastaseerde borstkanker met bepaalde risicofactoren

Het bestuderen van de incidentie en reversibiliteit van cardiotoxiciteit als gevolg van trastuzumab behandeling in 429 patiënten met HER2-positieve gemetastaseerde borstkanker heeft geleid tot een gericht advies met betrekking tot het monitoren van de cardiale functie. Op basis van de gevonden resultaten is seriële monitoring gedurende trastuzumab behandeling, bijvoorbeeld elke 3 maanden, geadviseerd in patiënten met HER2-positieve gemetastaseerde borstkanker die 1 of meer van de volgende risicofactoren hebben: LVEF van onder de 60%, eerdere cardiotoxiciteit gedurende neoadjuvante of adjuvante behandeling met trastuzumab en/of anthracycline of actief roken. Daar komt bij dat minder intensieve monitoring overwogen kan worden in patiënten zonder de gevonden relevante risicofactoren, vanwege de lage cumulatieve incidentie van ernstige cardiotoxiciteit in deze groep.

2. Een baseline LVEF van onder de 50% hoeft geen contra-indicatie te zijn voor het starten van trastuzumab behandeling

Zoals verwacht, hebben patiënten met HER2-positieve gemetastaseerde borstkanker en met een LVEF van tussen de 40-50% op baseline een hogere incidentie van cardiotoxiciteit als gevolg van de trastuzumab behandeling in vergelijking met patiënten met een LVEF van 50% en hoger. Desalniettemin, de cardiotoxiciteit in patiënten met LVEF van onder de 50% was grotendeels reversibel wat verklaard kan worden door tijdige behandeling met cardio-protectieve medicatie. Deze resultaten suggereren dat een baseline LVEF van onder de 50% niet een contra-indicatie hoeft te zijn voor het starten van trastuzumab behandeling en mogelijk meer patiënten met een verlaagde LVEF veilig behandeld kunnen worden met trastuzumab, wanneer er adequate cardio-protectieve zorg wordt geleverd. Echter, op dit moment is hier nog meer vervolgonderzoek naar nodig.

3. Het stoppen van trastuzumab na het bereiken van een rCR moet voorzichtig worden overwogen

Het afbouwen, of geheel stoppen van trastuzumab behandeling is van belang bij het reduceren van cardiotoxiciteit. In twee van de drie gevallen van patiënten die stopten met trastuzumab vanwege het bereiken van een rCR was er sprake van een langdurige

complete remissie van enkele jaren. Op basis van de resultaten gevonden in hoofdstuk 4 is het helaas nu niet mogelijk om te bepalen of het veilig is om trastuzumab te stoppen in alle patiënten die een rCR bereiken. Additioneel prospectief onderzoek is nodig om dit verder te exploreren. Voor nu is het nog steeds van belang om samen met de patiënt in een shared-decision making context te overwegen of trastuzumab gestopt kan worden na het bereiken van een rCR. Additioneel prospectief onderzoek is nodig om dit verder te exploreren.

4. Met het meten van GLS met 2DE-ST kan cardiotoxiciteit eerder en nauwkeuriger worden gedetecteerd
De huidige cardiale monitoring van patiënten met HER2-positieve borstkanker behandeld met trastuzumab wordt gedaan met de MUGA-scan of 2DE met of zonder ST. We toonden aan dat met het meten van de GLS door middel van 2DE-ST cardiotoxische veranderingen eerder en met grotere nauwkeurigheid kunnen worden voorspeld. Desondanks is er nog ruimte voor verbetering en zijn er meer studies nodig met grotere aantallen voordat 2DE-ST op grote schaal in de dagelijkse praktijk gebruikt kan worden.

5. Meer onderzoek is nodig naar de rol van NT-proBNP in het identificeren van cardiotoxiciteit
In **hoofdstuk 7** werd aangetoond dat patiënten met HER2-positieve borstkanker die cardiotoxiciteit ontwikkelden gedurende trastuzumab behandeling hogere NT-proBNP waardes en snellere toenames lieten zien. Desalniettemin, waren de veranderingen in NT-proBNP subtiel en was de klinische relevantie beperkt. Daarom concludeerden we dat enkel herhaalde metingen van NT-proBNP op dit moment niet geschikt is voor het adequaat detecteren van cardiotoxiciteit als gevolg van trastuzumab behandeling in patiënten met HER2-positieve borstkanker. Meer onderzoek is nodig naar de rol van NT-proBNP, alleen of in combinatie met andere biomarkers en naar de optimale afkapwaarde van NT-proBNP voor het accuraat identificeren van cardiotoxiciteit in patiënten met HER2-positieve borstkanker behandeld met trastuzumab.

6. STEMI-patiënten (met bepaalde risicofactoren) hebben een verhoogde kans op het ontwikkelen van kanker
Onderzoek naar de associatie tussen het ontwikkelen van een STEMI en kanker diagnose laat zien dat patiënten, ondanks zeldzaam, de eerste 3 maanden na een STEMI een sterk verhoogde kans hebben op kanker diagnose. Daarnaast is er een verhoogd risico op kanker diagnose in patiënten met verhoogde C-reactive protein (CRP) en trombocyten waardes en lagere hemoglobine waardes. Dit kan verklaard worden door het feit dat sommige STEMI's mogelijk van paraneoplastische origine zijn. Verder hebben we onze gevonden resultaten gecorrigeerd voor gedeelde risicofactoren tussen kanker en STEMI

zoals leeftijd, geslacht, roken, diabetes, body mass index (BMI) en CRP. Desondanks kan residuele confounding niet worden uitgesloten. Ondanks dat de etiologie tussen STEMI en kanker nog niet geheel duidelijk is, is het goed om bewust te zijn van het verhoogde risico op kanker diagnose in STEMI-patiënten, en met name in patiënten die bij STEMI presentatie hogere CRP waardes, trombocyten en leukocyten aantallen, en/of lagere hemoglobine waardes hebben.

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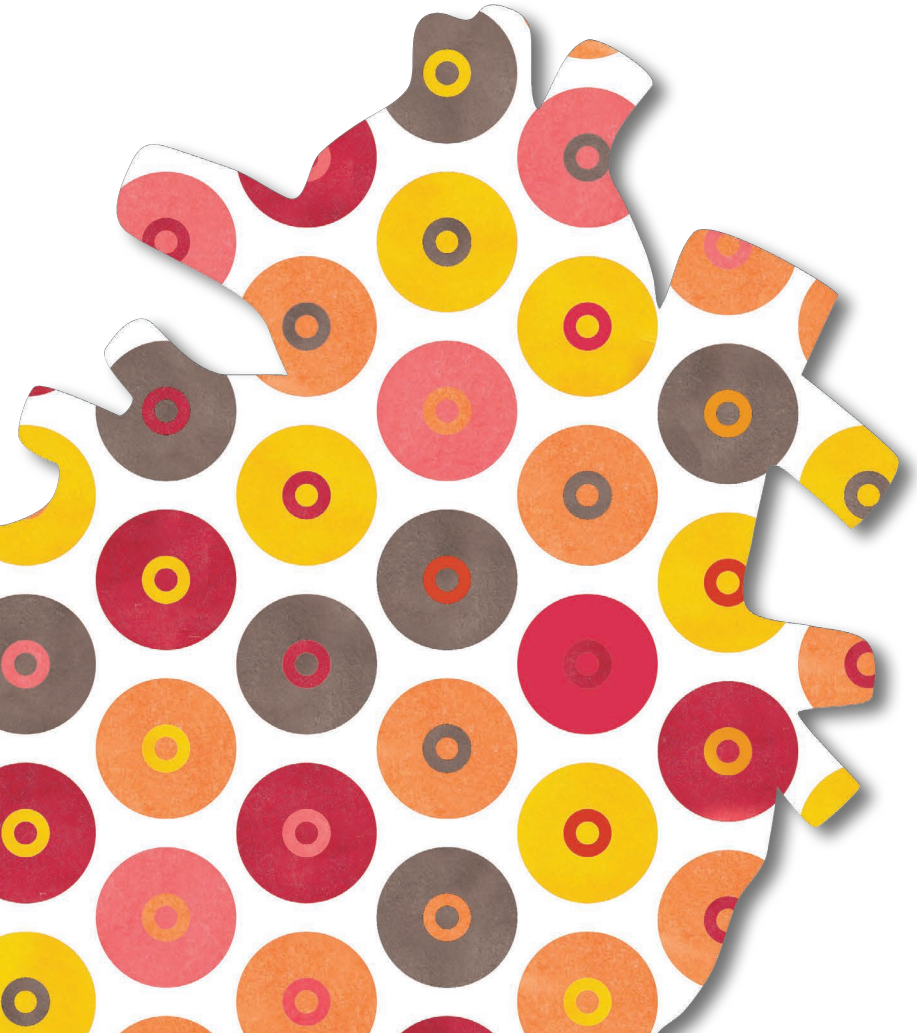

EPILOGUE

LIST OF PUBLICATIONS

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1. **Bouwer NI**, Liesting C, Kofflard MJM, Sprangers-van Campen SM, Brugts JJ, Kitzen JJEM, Fouraux MA, Levin MD, Boersma E. NT-proBNP correlates with LVEF decline in HER2-positive breast cancer patients treated with trastuzumab. Cardiooncology. 2019 May 28; 5:4.
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3. **Bouwer NI**, Jager A, Liesting C, Kofflard MJM, Brugts JJ, Kitzen JJEM, Boersma E, Levin M-D. Cardiac monitoring in HER2-positive patients on trastuzumab treatment: A review and implications for clinical practice. Breast. 2020 Aug; 52:33-44.
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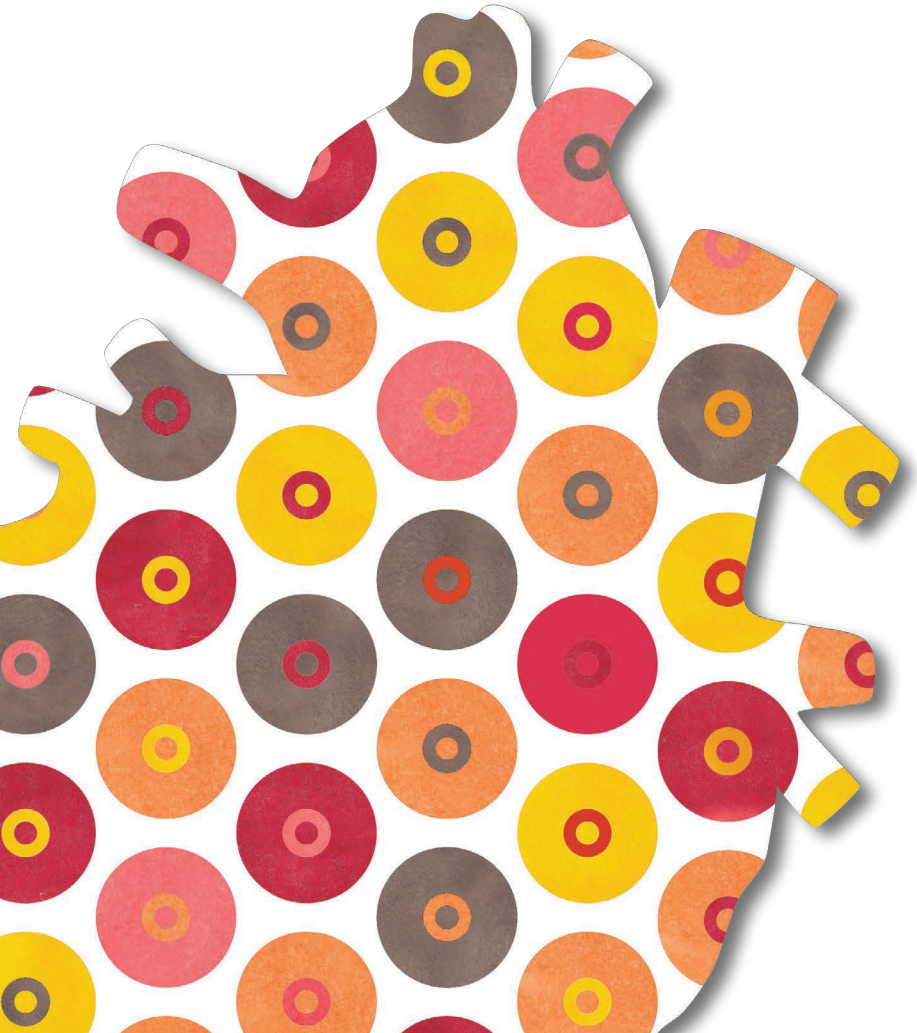
6. **Bouwer NI**, Liesting C, Kofflard MJM, Brugts JJ, Kock MCJ, Kitzen JJEM, Levin MD, Boersma E. 2D-echocardiography vs cardiac MRI strain: a prospective cohort study in patients with HER2-positive breast cancer undergoing trastuzumab. Cardiovasc Ultrasound. 2021 Nov 9;19(1):35.
7. **Bouwer NI**, Steenbruggen TG, Rier HN, Kitzen JJEM, Smorenburg CH, van Bekkum ML, de Jong PC, Drooger JC, Holterhues C, Kofflard MJM, Boersma E, Sonke GS, Levin MD, Jager A. The effect of trastuzumab on cardiac function in patients with HER2-positive metastatic breast cancer and reduced baseline left ventricular ejection fraction. Int J Cancer. 2022 Apr 11.

Submitted manuscripts

8. **Bouwer NI**, Leening MJG, Ikram MA, RIkje Ruiter T, Kavousi M, Boersma E, Kofflard MJM, Van den Bos E-J, Weevers APJD, Deckers JW, Levin M-D. Risk of cancer after ST-segment-elevation myocardial infarction.



EPILOGUE

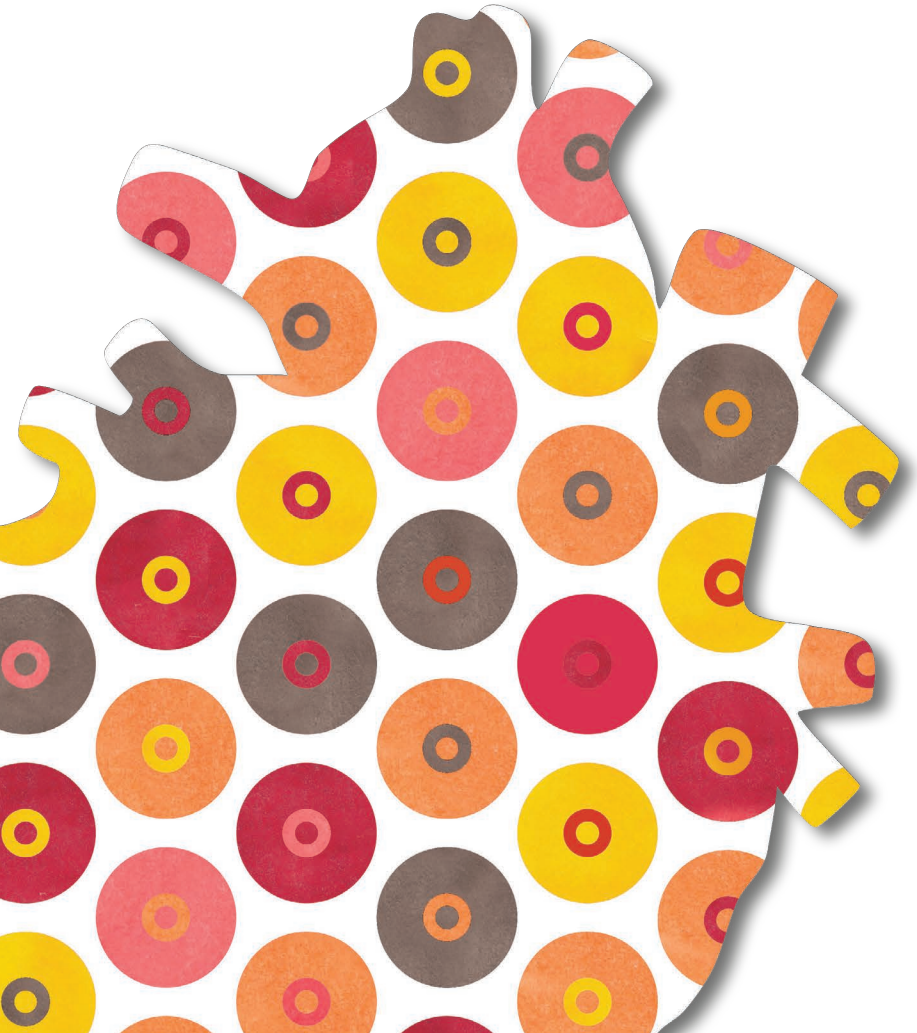
PHD PORTFOLIO

PHD PORTFOLIO

PhD training	Year	Workload (ECTS)
General courses		
Good clinical practice	2017	0.5
Scientific Writing in English	2018	1
Scientific Integrity in Research	2019	0.3
CPO (Consultation Center for Patient Oriented Research)	2018	0.3
In-depth courses		
Joint modelling (NIHES)	2018	0.7
Practice of Epidemiological analysis, (NIHES)	2018	0.7
Biostatistical Methods IL Basic principles (NIHES)	2018	5.7
Planning and Evaluation of Screening, (NIHES)	2019	1.4
Repeated Measurements (NIHES)	2019	1.7
Cancer Epidemiology (NIHES)	2019	1.4
Missing Values (NIHES)	2019	1.7
Cardiovascular Epidemiology (NIHES)	2019	0.9
Introduction to Epidemiology (NIHES)	2019	0.7
Clinical Trials (NIHES)	2019	0.7
Health Economics (NIHES)	2019	0.7
Fundamentals of Medical Decision Making (NIHES)	2019	0.7
Methods of Public Health Research (NIHES)	2019	0.7
Gender and Research (NIHES)	2019	0.7
Study Design (NIHES)	2019	4.3
Clinical Translation of Epidemiology (NIHES)	2019	2.0
Clinical Epidemiology (NIHES)	2019	3.7
Principles in Causal Inference (NIHES)	2019	1.4
Biostatistical Methods II: Classical Regression Models (NIHES)	2019	4.3
Competing Risks and Multi-State Models (NIHES)	2020	0.9
Advanced Clinical Trials (NIHES)	2020	1.9
Advanced Analysis of Prognosis Studies (NIHES)	2020	1.9

PhD training	Year	Workload (ECTS)
Quality of Life Measurements (NIHES)	2020	0.9
Preventing Failed Interventions (NIHES)	2020	1.4
Psychiatric Epidemiology (NIHES)	2020	1.1
Social Epidemiology (NIHES)	2020	0.7
The Placebo Effect (NIHES)	2020	1.4
Seminars and workshops		
Enhancing Precision Medicine Through Protein Biomarker Profiling	2017	0.3
Cardio-Oncology (UMCU)	2017	0.3
Sex and Gender in Cardiovascular Research (COEUR)	2018	0.5
Pathophysiology of Ischemic Heart Disease (COEUR)	2018	0.5
Solving the Mysteries of Atrial Fibrillation (COEUR)	2018	0.3
Heart Failure Research (COEUR)	2018	0.5
Imaging for Ischemic Heart and Brain Disease (COEUR)	2018	0.5
Vascular Clinical Epidemiology (COEUR)	2019	0.4
PhD day (COEUR)	2019	0.4
Heart Transplantation (COEUR)	2019	0.4
Networked Sciences	2019	0.5
Sex and Gender in Cardiovascular Research (COEUR)	2019	0.5
Advanced Decision Making in Vascular Care (COEUR)	2020	0.5
Cardio-Oncologie (CVOI)	2020	0.3
4th translational Cardiovascular Research Meeting	2020	0.3
Borstkanker Behandeling Beter	2019 – 2020	1.0
NABON-BOOG symposium	2020	2.0
Congenital Cardiology (COEUR)	2021	0.5
Oral Presentation		
Internistendagen	2019	1.2
Wetenschapsdag Albert Schweitzer Hospital	2019	1.2
Wetenschapslunch Albert Schweitzer Hospital	2019	1.2

PhD training	Year	Workload (ECTS)
NVVC Najaarscongres	2020	1.2
MedTalks: SABCS Journaal	2020	1.2
Borstkanker Research Meeting Erasmus MC	2021	1.2
Wetenschapsdag Albert Schweitzer Hospital	2021	1.2
Poster Presentation		
ESC Heart Failure	2019 - 2020	1.2
ESMO Virtual Congress	2020	0.6
EACVI Best of Imaging, Virtual Congress	2020	0.6
San Antonio Breast Cancer Congress	2020	0.6
ACC/AHA meeting	2021	0.6
International conferences		
ESC Heart Failure	2019 - 2020	1.5
ESMO Virtual Congress	2020	0.7
ASCO Virtual Congress	2020	0.7
HFA Discoveries	2020	0.7
EACVI Best of Imaging, Virtual Congress	2020	0.7
San Antonio Breast Cancer Congress	2020	0.7
ACC/AHA meeting	2021	0.6
Teaching		
Supervising 2nd year medical students *Subject: Long-term effects of trastuzumab treatment of the cardiac function of patients with HER2-positive breast cancer*	2018 – 2019 2019 – 2020 2020 – 2021	2.5
Other		
Peer review for several international journals in the field of Cardio-Oncology	2019 – 2021	0.7



EPILOGUE

ABOUT THE AUTHOR

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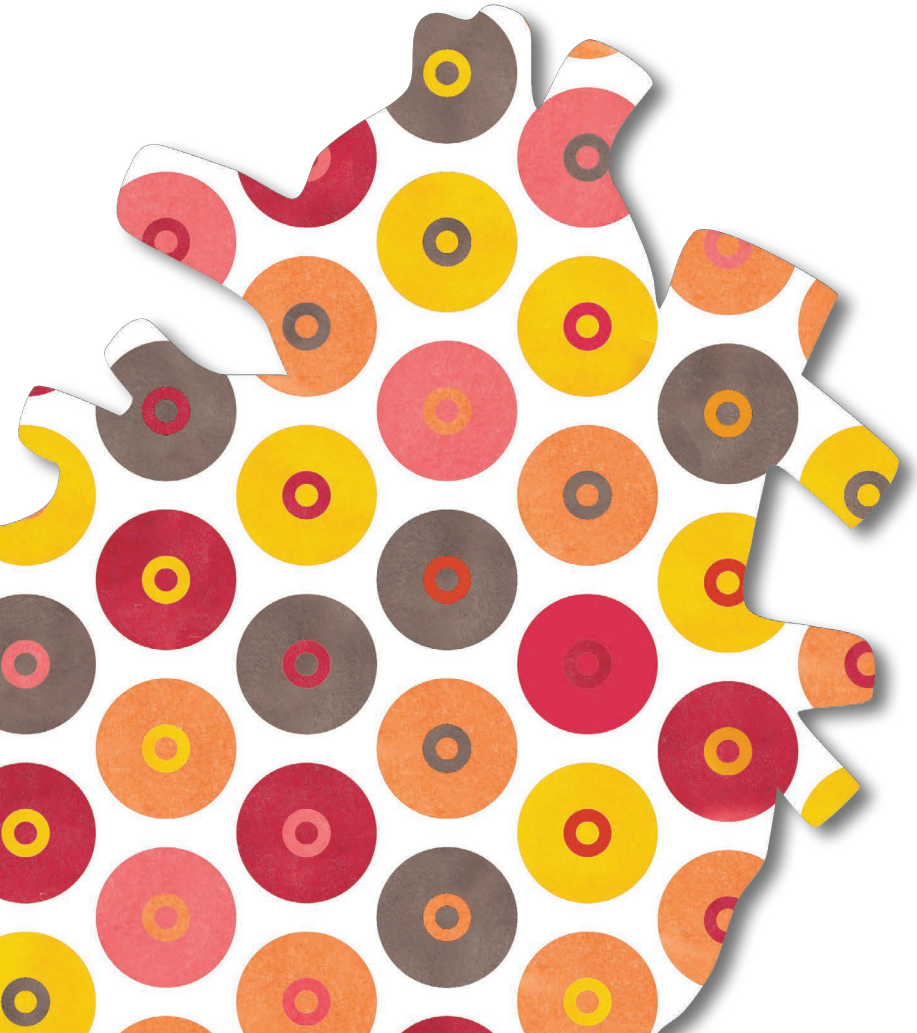
Nathalie Ilonka Bouwer was born on April 23rd, 1995, in Rotterdam, the Netherlands. She graduated from secondary education (Gymnasium) cum laude in Barendrecht, the Netherlands, in 2013.

Thereafter, she started medical school at the Erasmus Medical Centre, Rotterdam, The Netherlands. After obtaining her bachelor's degree in Medicine in 2016, she started working on a research project in the Albert Schweitzer Hospital in Dordrecht as part of the Master programme of Medicine. She finished this research project in 2017, after which she continued her research as a PhD candidate working in the Albert Schweitzer Hospital under close supervision of prof. dr. ir. Eric Boersma on cardiac monitoring of patients with HER2-positive breast cancer during trastuzumab treatment. In addition, in 2019 she enrolled in the research master Health Science with field of expertise Clinical Epidemiology at the Netherlands Institute for Health Sciences (NIHES), Rotterdam, which she successfully finished in the winter of 2022. Nathalie will graduate from medical school in the summer of 2023.

Nathalie Ilonka Bouwer werd geboren op 23 april 1995 te Rotterdam. In 2013 voltooide zij het Gymnasium Cum Laude aan het Dalton Lyceum Barendrecht. In hetzelfde jaar begon zij aan de studie Geneeskunde aan het Erasmus Medisch Centrum in Rotterdam. Nadat zij haar bachelor Geneeskunde behaalde in 2016, deed zij onderzoek in het Albert Schweitzer Ziekenhuis in Dordrecht als onderdeel van de Master Geneeskunde. Dit onderzoek werd in 2017 afgerond, waarna zij haar onderzoek vervolgde in het Albert Schweitzer Ziekenhuis onder begeleiding van prof. dr. ir. Eric Boersma over cardiale monitoring van HER2-positieve borstkanker patiënten gedurende trastuzumab behandeling. Daarnaast, begon zij in 2019 aan de Research Master Health Science met specialisatie Clinical Epidemiology bij het Netherlands Institute for Health Sciences (NIHES), Rotterdam, welke zij succesvol afrondde in de winter van 2022. Nathalie zal in de zomer van 2023 afstuderen als arts.



EPILOGUE

DANKWOORD

DANKWOORD

Dankbaar ben ik voor alle hulp, begeleiding en betrokkenheid bij de totstandkoming van dit proefschrift. Terwijl op de voorkant van dit proefschrift maar 1 naam vermeld staat, realiseer ik mij ten volle dat dit proefschrift er niet zou zijn geweest en niet hetzelfde was geweest zonder de inspanningen van velen. Zonder maar iemand tekort te willen doen, zou ik graag hierbij een aantal mensen in het bijzonder bedanken.

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Tijdens dit promotietraject heb ik mooie mensen leren kennen die het hele traject wat lichter en vooral veel gezelliger hebben gemaakt. Graag begin ik met alle dames uit kamer W04.046 in het Albert Schweitzer ziekenhuis in Dordrecht: **Inge**, jij was bijna klaar toen ik begon, maar ik heb zeker nog 2 maanden van jouw positivisme kunnen genieten en ik geloof ook heilig dat het afplakken van de datum op je computer echt helpt bij een naderende deadline (geloof me, ik heb het ook geprobeerd deze laatste maanden..). **Martine**, alhoewel het lijkt dat we niet van elkaar afkomen (eerst ging ik weg en kwam ik weer terug, en toen ging jij weg en kwam jij weer terug), lijkt het toch echt dat we de komende tijd niet meer samen op een kamer zullen zitten. Dank voor alle gezelligheid en sportieve rondjes. **Hanah**, bedankt voor jouw introductie in het onderzoek doen, de begeleiding en de gezellige praatjes over de jaren! **Annemarie**, als collega-promovendus uit het Albert Schweitzer Ziekenhuis deelden we naast veel tijd, ook veel frustraties samen. Fijn dat het uiteindelijk voor allebei van ons goed is afgelopen en veel succes nog met je opleiding tot huisarts! **Lina**, als collega-promovendus maar ook als collega-student Geneeskunde hield jij mij op de hoogte van al je coschap avonturen, waar ik nu ook vol in zit. We stonden namelijk allebei voor dezelfde keuze, en jij besloot om eerst met je coschappen te beginnen en daarna (lees tegelijkertijd) te promoveren. Vol bewondering heb ik aanschouwd hoe jij dit hebt gecombineerd en hopelijk kan ik jou nog verblijden met goede coschappen verhalen! **Camille**, als Vlaamse onderzoekster kwam je Dordrecht verblijden met een flinke dosis humor en goede muzieksmaak! Ik luister nog steeds vaak naar de lofi hiphop playlist en ik ben niet vergeten dat we nog eens samen de hele marathon in Rotterdam of een gedeelte van een triatlon gaan doen. Bedankt dat je op de grote dag als paranimf aan mijn zijde wilt staan! **Paul**, een vaste kracht op de vrijdag in Dordrecht verdiend natuurlijk ook een speciale vermelding, zeker omdat jij er op een gegeven moment vaker aanwezig was dan ik vanwege alle cursussen. Veel succes met de rest van je onderzoek! **Martijne**, ik twijfelde of ik je bij het Albert Schweitzer of Erasmus MC moest zetten want ik kwam je in allebei de ziekenhuizen tegen. Ik vergeet nooit meer het etentje om mijn eerste publicatie te vieren waarbij we (lees jullie) voor het hoofdgerecht al buiten ATLS aan het

verrichten waren. Hopelijk verlopen niet al jouw etentjes zo.. Ook noem ik nog graag de rest van de kamer die mij in de onderzoek tijd met de nodige aantallen mokken thee en gezellige praatjes hebben verzorgd: **Sandra**, **Maaike**, **Claudia**, **Riëlla**, **Miranda**, **Rosita**, **Sylvia** en **Silvia**, waarvoor dank! Niet uit kamer W04.046, maar wel hier vaak te vinden voor een gezellig praatje meer of minder: **Sara**, ik ben zeer benieuwd of er gedurende jouw lekenpraatje ook vakantiefoto's te zien zullen zijn!

Natuurlijk waren er in het Erasmus MC ook hele leuke onderzoek collega's die hebben gezorgd dat de jaren voorbijvlogen. Ten eerste de KLEP-groep: **Elke**, heel erg bedankt voor het warme welkom in de KLEP-groep en het gebruiken van je bureau en computer op Rg4 elke woensdag de afgelopen jaren. Ondanks je drukke poli's en strikte deadlines, was jij altijd in voor een kletspraatje, koffiepauze of borrel. Ik ben blij dat het Dordrecht virus ook bij jou is overgeslagen, veel geluk daar nog! **Anne-Sophie**, wat begon als een gezellig idee om Elke te vergezellen in haar hele marathon uitdaging, werd een uitdaging voor onszelf waarbij we samen als duo de hele marathon gingen lopen. Bedankt voor die raketten die je via Whatsapp stuurde rond kilometer 18 want anders had je misschien nu nog steeds daar op het wisselpunt op mij staan wachten.. **Marlou**, mijn trouwe cursus medegenoot! Wat heb ik met je kunnen lachen tijdens de verschillende NIHES-cursussen. Hopelijk duurt het vieren van dit proefschrift met Koekela minder lang dan het vieren van onze laatste cursusdag met Koekela.. Haal jij dit keer?! Binnenkort ook weer snel een rondje samen op de racefiets! **Muhammed**, **Michelle en Marie**, het eind is in zicht, succes nog met de laatste loodjes! **Maxime** & **Victor**, bedankt dat ik helemaal in het begin een paar maanden 1 dag per week bij jullie in de zusterflat, ook wel "de sauna" of "het Z-gebouw" genoemd, heb mogen zitten. Ik heb genoten, zeker in de maanden juli en augustus. Daarnaast waren jullie (al bijna) klaar toen ik begon, maar dit nam niks af aan jullie betrokkenheid en sfeer aan de KLEP-groep: **Victor**, **Jan-Kees** en **Nermina**, het gaat jullie goed! Vanzelfsprekend wil ik alle andere onderzoek collega's van de Cardiologie hier nog expliciet noemen: **Carlijn**, **Frederike**, **Laurie**, **Jesse**, **Roy**, **Savine**, **Karishma**, **Stefan** en **Sumant** en iedereen die ik nu nog vergeet: bedankt voor de gezelligheid op de congressen en borrels.

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of toch beter een zonvakantie?! Bedankt dat jij op de grote dag als ondersteunende paranimf naast mij staat! Je was zo vaak aan het vissen naar mijn paranimfen dat ik je bijna niet niet kon vragen! Wie had dat gedacht toen we al die jaren terug ons profielwerkstuk samendeden over de invloed van kleur op het kortetermijngeheugen dat we zo direct samen daar mogen staan. Samen ook begonnen aan Geneeskunde, maar gelukkig heb jij je eigen draai gevonden in iets wat veel beter bij je past. Niet alleen op werk, maar ook privé sta je altijd voor iedereen klaar en is niks of geen enkel kleinigheidje jou te veel. **Marjolein,** nu we eindelijk binnen een straal van 5 kilometer van elkaar wonen worden de gezellige stads-en parkwandelingen en avonden voetbal kijken veel gemakkelijk ingepland, wat ik erg leuk vind! Ik kijk uit naar nog meer van dit. Hetzelfde geldt voor **Marieke**, wat leuk dat je ook wat dichterbij bent komen wonen. Zijn die avondjes ploeteren met je laminaat toch niet voor niks geweest. Met je ontzettende fanatieke houding ben je een welkom onderdeel van al onze spelletjesavonden waarvan ik vind dat we echt nog wel vaker kunnen houden! En **Renée**, dan weer in Leiden, Zweden, Duitsland en dan weer in Zweden! Ik vind het erg stoer hoe vaak jij het avontuur (vaak in het buitenland) durft aan te gaan, en dat er voor jou soms geen enkele beren op de weg lijken te zijn. Als collega-onderzoeker wil ik je veel succes wensen met jouw promotieonderzoek en ik kom sowieso snel weer jouw kant op voor fika en natuurlijk ook om met Noa te knuffelen. **Maaike**, wat was ik blij met jou als navigator in Ierland aan de verkeerde kant van de weg, en dan vooral ook bij het nemen van de rotondes. Ik zweette me dus ook de peentjes toen je op een gegeven dus zo erg in Ierland vast zat dat we je bijna niet uit het moeras getrokken gekregen.. Gelukkig is het allemaal goed gekomen, met ook maar minimale schade aan de auto van dat ene stoeprandje. **Nicky** en **Sofie**, ook al spreek ik jullie wat minder vaak, toch zijn jullie ook een erg belangrijk onderdeel in de groep en mogen jullie dus ook niet vergeten worden.

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