Diplomarbeit

Association of Trastuzumab (Herceptin)-induced Cardiotoxicity with co-morbid Disease and Pharmacotherapy in Breast Cancer Patients

eingereicht von

Saskia Fiona Firla

zur Erlangung des akademischen Grades

Doktor(in) der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt an der

Universitätsklinik für Innere Medizin

Klinische Abteilung für Kardiologie und

Klinische Abteilung für Onkologie

unter der Anleitung von

Assoz.-Prof. Priv.-Doz. Dr.med. Dr.scient.med. Peter Rainer und

Assoz. Prof. Priv.-Doz. Mag. Dr.med.univ. Martin Pichler

Graz, 02.05.2018

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 02.05.2018 Saskia Fiona Firla eh

Acknowledgements

First of all, I want to thank my supervisor Dr. Peter Rainer not only for providing the topic but especially for the encouragement, critical advice and the patience in overseeing this thesis. I would also like to thank my second supervisor Dr. Martin Pichler.

Special thanks go to my entire family and particularly to my parents. Thank you for the continuous support and for everything that you have done for me!

Last but not least I want to thank my friends for always being there with help and advice and for making the past years an amazing time!

Zusammenfassung

Einleitung:

Brustkrebs ist die weltweit häufigste Krebserkrankung bei Frauen. In ca. 20% der Fälle liegt eine HER2-Überexprimierung vor, welche ursprünglich mit einer schlechteren Prognose assoziiert war. Heutzutage steht mit dem monoklonalen Antikörper Trastuzumab eine wirkungsvolle Therapie zu Verfügung, deren Einsatz jedoch in vielen Fällen durch das Auftreten kardiotoxischer Nebenwirkungen limitiert ist. Obwohl einige Risikofaktoren bekannt sind, gibt es keinen Konsens über die Wirksamkeit kardioprotektiver Medikation zur Prävention der kardialen Dysfunktion. Ziel dieser Arbeit ist es, einen Überblick über das Auftreten von Kardiotoxizität bei einer Patientengruppe am LKH Graz zu geben sowie Vorerkrankungen und Begleitmedikation in dieser Kohorte und deren möglichen Zusammenhang mit Kardiotoxizität zu analysieren.

Material und Methoden:

Bei der Studie handelt es sich um eine monozentrische retrospektive Studie. Eingeschlossen wurden 202 Patientinnen mit invasivem Mammakarzinom, welche zwischen 2004 und 2016 eine Therapie mit Trastuzumab an der Klinik für Onkologie am LKH Graz begannen. Erhobene Daten wurden mittels deskriptiver Statistik ausgewertet und graphisch dargestellt. Anhand des Auftretens von Kardiotoxizität wurden zwei Gruppen gebildet, welche mittels induktiver Statistik verglichen wurden.

Ergebnisse:

Kardiotoxische Nebenwirkungen führten zu einem Therapieabbruch in 11.9% der Fälle. Ein relevanter LVEF-Abfall konnte bei 12.4% der Patienten festgestellt werden. Diese Patienten hatten signifikant höhere Werte für linksventrikuläre end-diastolische Dimensionen zu Therapiebeginn und hatten signifikant häufiger ACE-Hemmer als Begleitmedikation. In der Gruppe mit Kardiotoxizität konnten ein höheres Alter, ein höherer BMI und eine niedrigere LVEF zu Therapiebeginn beobachtet werden, diese Ergebnisse waren jedoch nicht signifikant. Arterielle Hypertension war häufiger in der Gruppe mit Kardiotoxizität zu beobachten, wohingegen Rauchen dort seltener beobachtet wurde und sich die Raten für Diabetes mellitus und Hypercholesterinämie in beiden Gruppen glichen. Weiters konnten eine erhöhte Rate an Calciumantagonisten, sowie erniedrigte Raten von Statinen und Spironolacton in der Gruppe mit Kardiotoxizität

beobachtet werden. Kein relevanter Unterschied bestand bezüglich Sartanen, Betablockern, anderen Diuretika, Diabetesmedikation und Nitraten. Keine dieser Beobachtungen bezüglich der Begleitmedikation und Begleiterkrankungen war jedoch statistisch signifikant.

Zusammenfassung:

Patienten mit Kardiotoxizität zeigten höhere links-ventrikuläre end-diastolische Dimensionen zu Therapiebeginn sowie eine höhere Rate an Begleitmedikation mit ACE-Hemmern. Unterschiede zwischen beiden Gruppen bezüglich Alter, BMI, der Ausgangs-LVEF sowie der Rate an weiteren Risikofaktoren und Begleitmedikation waren statistisch nicht signifikant.

Abstract

Introduction:

Breast cancer is the most common cancer in women. In about 20% of cases an overexpression of the HER2 receptor can be found, which was formerly associated with a poorer outcome. Nowadays the monoclonal antibody trastuzumab presents an effective therapy option. However, its clinical use is limited by the occurrence of cardiac dysfunction. Although some risk factors are known, studies on the effect of cardioprotective therapy as a mean of prevention have delivered inconclusive results. Aim of this study is to give an overview of the incidence of cardiotoxicity in the patient group treated at the LKH Graz and to analyze possible associations between the occurrence of cardiotoxicity and co-morbid disease and co-therapy.

Methods:

We conducted a single-center retrospective study with invasive breast cancer patients who received trastuzumab treatment at the department of oncology at the university hospital in Graz between 2004 and 2016. 202 female patients were included in the final study. Collected data was analyzed using descriptive statistics and presented graphically. Based on the occurrence of cardiotoxicity two groups were distinguished whose parameters were compared using inductive statistics.

Results:

Treatment with trastuzumab was discontinued due to cardiotoxic side effects in 11.9% of patients and a relevant LVEF decrease could be observed in 12.4% of patients. Those patients had significantly higher baseline LVED dimensions and were significantly more often on ACE-inhibitor co-medication. Patients with cardiotoxicity were older, had a higher BMI and lower baseline LVEF, although these results failed to reach significance. Arterial hypertension was more frequent in the cardiotoxicity group, whereas smoking was less common. Rates for diabetes and hypercholesterolemia were similar in both groups. A higher rate of calcium channel blockers and a lower rate of statins and spironolactone could be observed in the cardiotoxicity group. Further cardiovascular medication was similar among both groups. None of these observations regarding co-morbid disease and co-therapy was statistically significant.

Conclusion:

Patients with cardiotoxicity had higher baseline LVEDD values and were on medication with ACE-Inhibitors more frequently. Differences between both groups regarding age, BMI, baseline LBEF and smoking rate and frequency of statin-co-medication were not statistically significant.

Table of Contents

Glossary and Abbreviations

2D	Two-dimensional
5-FU	Fluorouracil
ACEIs	Angiotensin-converting-enzyme inhibitor
ADCC	Antibody-dependent cell-mediated cytotoxicity
Akt	Protein kinase B
ARBs	Angiotensin II receptor blockers
ASE	American Society of Echocardiography
BAX	Apoptosis regulator BAX
BBs	Beta blockers
BMI	Body mass index
BRCA1/2	Breast cancer 1/2
BTC	Betacellulin
CRP	C-reactive protein
EACVI	European Association of Cardiovascular Imaging
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMA	European Medicines Agency
EPR	Epiregulin
ER	Estrogen receptor
ErbB2	Human epidermal growth factor receptor 2
Erk	Extracellular signal-regulated kinases (=MAPK)
ESC	European Society of Cardiology
FDA	Food and Drug Administration
Gal	Galectin
GDF	Growth differentiation factor
HB-EGF	Heparin-binding EGF-like growth factor
HCT	Hydrochlorothiazide
HER2/neu	Human epidermal growth factor receptor 2
hs-TnT/I	High-sensitive troponin T/I
iPCS	Induced pluripotent stem cell
KPS	Karnofsky Performance Score
LKH Graz	Landeskrankenhaus Graz / University Hospital Graz
LV	Left ventricular

LV2D	2D left ventricular end-diastolic dimension
LVEDD	Left ventricular end-diastolic dimension
LVEDVi	Left ventricular end-diastolic volume index
LVEF	Left ventricular ejection fraction
LVF	Left ventricular function
M	Mean
MAPK	Mitogen-activated protein kinase (=ERK)
MPO	Myeloperoxidase
MRI	Magnetic resonance imaging
m-Tor	Mechanistic target of rapamycin
MUGA	Multigated acquisition scan
NRG	Neuregulins
NT-proBNP	NT-pro Brain natriuretic peptide
PI3K	Phosphoinositide 3-kinase
PlGF	Placental growth factor
PR	Progesterone receptor
ROS	Reactive oxygen species
SD	Standard deviation
sFlt	Soluble fms-like tyrosine kinase-1
TGF-α	Transforming growth factor alpha
top	Topoisomerase
UICC	Union for International Cancer Control
WHO	World Health Organization

List of Figures

List of Tables

1 Introduction

1.1 Background and Aim of the Study

Breast cancer is the most common type of cancer in women worldwide.(1) According to the National Institute of Cancer 12.4 percent of women – meaning one in eight women – in the United States will be affected during their lifetime.(2) Advances in cancer therapy and screening have led to a reduction of mortality during the past years.(3) However, this success in therapy also leads to a growing number of surviving patients that must live with long-term effects caused by antineoplastic therapy.(4) Studies have shown that in breast cancer survivors the risk for cardiovascular disease-related mortality is higher compared to women without breast cancer. Heart disease has been shown to be the greatest single non-cancer related cause auf death in female breast cancer patients.(5,6) In about 20% of breast cancer patients HER2/neu is amplified.(7,8) In these cases, trastuzumab is an important treatment option. Trastuzumab however is known to cause cardiotoxicity.(9) This presents one of the most important adverse effects and often is a limiting factor in the use of this therapy, which consequently leads to a higher risk of cancer reoccurrence and mortality.(10)

Although some risk factors have been described there is still no common agreement on how to prevent cardiotoxicity. The studies on preventive effects of cardiovascular medication that have been published delivered inconclusive results. Therefore, more studies are needed to get a clear understanding of this topic. Aim of this retrospective study is to find out more about the association of trastuzumab-induced cardiotoxicity with co-morbid diseases and pharmacotherapy in a group of breast cancer patients treated at the University Hospital of Graz. More precise knowledge of risk factors and especially protective factors of trastuzumab-induced cardiotoxicity would lead to a safer and more effective use of this drug. Restricted medical resources could be used more efficiently if women at high risk would receive closer monitoring while women at low risk could profit from not having unnecessary examinations. Given the relatively high incidence of breast cancer this would also have economic effects. More importantly however, it would significantly improve the health of a great number of women worldwide.

1.2 Breast Cancer

1.2.1 Epidemiology

In the year 2012 about 1.67 million women worldwide have been diagnosed with breast cancer. This number makes up 25% of all cancer diagnoses in women for that year, meaning that breast cancer is the most common type of cancer in women overall both in developed and less developed countries. Breast cancer is the number one cause of cancer death in women in less developed countries and it is number two after lung cancer in developed countries. In 2012 there was a total number of 522,000 breast cancer deaths worldwide.(1)

In Austria, 5,390 women have been diagnosed with breast cancer in 2015. This number makes up about 30% of all new female cancer cases that year. Breast cancer was the number one cause of cancer death in women in 2015 and at the end of the year there were 74,170 women with breast cancer living in Austria.(11)

During recent years there have been great advances in cancer therapies leading to a reduction in breast cancer mortality.(12) The relative 5-year survival rate of women diagnosed with breast cancer in Austria during the years 2008-2012 is 86.3% compared to 72.6% for women diagnosed between 1988-1992.(13)

Figure 1 gives an overview of the incidence and the mortality of breast cancer during the years 1990 and 2014.

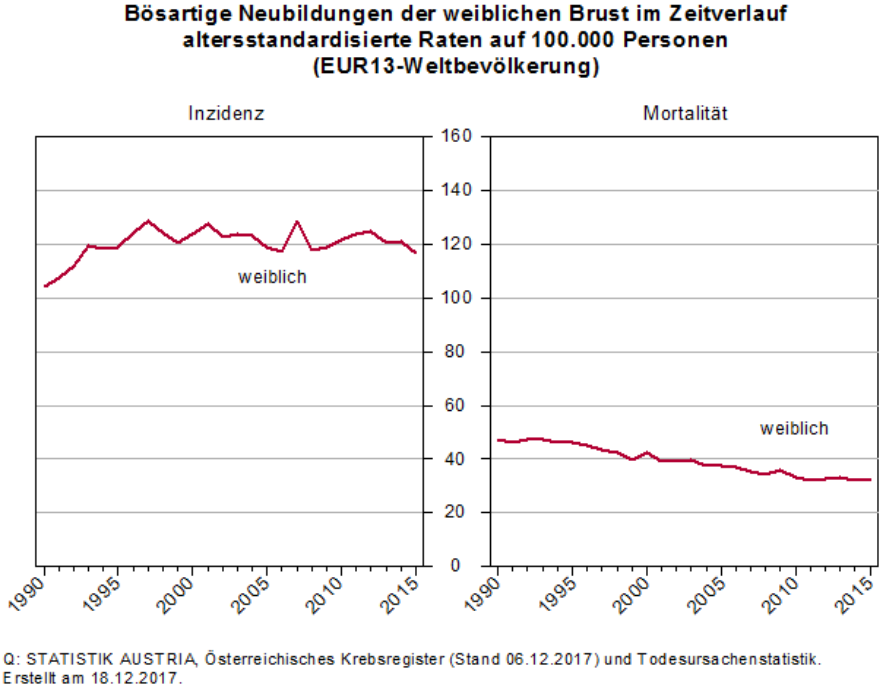

Figure 1: Breast cancer incidence and mortality from 1990 to 2015. Derived from (14)

1.2.2 Etiology and Risk Factors

Multiple factors contribute to the development of breast cancer. Risk factors can be genetic predisposition, hormonal or environmental influences and include:(15)

- Female gender
- Age
- Positive family history in a first degree relative, BRCA1/2
- Early menarche, late menopause
- Nulliparity or older age (>35 years) at first child birth
- Oral contraceptives, hormone replacement therapy
- Lifestyle factors (excessive alcohol consumption, fatty diet)

1.2.3 Classification, Staging and Grading

In 2012 the World Health Organization (WHO) published an updated classification of tumors of the breast according to their histopathology.(16–18) The classification for invasive carcinomas is presented in the **table 1**.

Invasive breast carcinomas
Invasive carcinoma of no special type (NST)
Invasive lobular carcinoma
Tubular carcinoma
Cribiform carcinoma
Mucinous carcinoma
Carcinoma with medullary features
Carcinoma with apocrine differentiation
Carcinoma with signet ring differentiation
Invasive micropapillary carcinoma
Metaplastic carcinoma of no special type
Rare types

Table 1: WHO classification of invasive breast carcinomas 2012. Modified after (16–18)

Staging of breast cancer takes place according to the TNM classification, in which the size and extent of the primary tumor (T), regional lymph nodes (N) and metastasis (M) are

evaluated.(19) Stages according to the Union for International Cancer Control (UICC) are presented in **table 2**.

Breast cancer stages according to the UICC			
Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T0, T1	N1mi	M0
Stage IIA	T0, T1 T2	N1 N0	M0 M0
Stage IIB	T2 T3	N1 N0	M0 M0
Stage IIIA	T0, T1, T2 T3	N2 N1, N2	M0 M0
Stage IIIB	T4	N0, N1, N2	M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	Any N	M1

Table 2: Breast cancer staging according to UICC 8th edition. Modified after (10)

Breast carcinoma grading usually takes place according to the Nottingham grading system. Here G1 describes a low grade or well differentiated tumor, G2 an intermediate grade or moderately differentiated and G3 a high grade or poorly differentiated tumor.(20) Additionally, breast cancer can be further categorized according to the molecular subtype. Important aspects in this case are the hormonal receptor status (estrogen and progesterone receptor positivity or negativity), the proliferation index Ki67 and the Her2/neu status.(21) Depending on the Ki67 value tumors have been classified as low (≤15%), intermediate (16-30%) and highly proliferating (>30%) by the St. Gallen expert consensus in 2009.(22) HER2-status is determined using immunohistochemistry (IHC) or in-situ hybridization (ISH). Requirement for initiation of trastuzumab therapy is a 3+ IHC result or a positive ISH result. In case of a 2+ IHC result, the tests have to be repeated either with IHC at different tissue samples or ISH. IHC 0 and 1+ are considered negative.(23–25) Further information on criteria of the single categories is given in **table 3**.

Method	Criteria
IHC	
0	No or faint incomplete membrane staining in <10% of tumor cells
1+	Faint incomplete membrane staining in >10% of tumor cells
2+	Weak/moderate circumferential membrane staining in >10% of tumor cells or intense circumferential membrane staining in <10% of tumor cells
3+	Complete and intense circumferential membrane staining in >10% of tumor cells
ISH	
Negative	HER2/CEP17 ratio <2.0 and average HER2 copy number <4 signals/cell
Equivocal	HER2/CEP17 ratio <2.0 and average HER2 copy number <6 and ≥4 signals/cell
Positive	HER2/CEP17 ratio ≥2.0 or average HER2 copy number ≥6 signals/cell

Table 3: Criteria for immunohistochemistry and in-situ hybridization positivity. **Modified after (23–25).** IHC= immunohistochemistry, ISH= in-situ hybridization

1.2.4 Therapy

There is no uniform treatment for breast cancer. The exact therapy regimen varies according to tumor identity, receptor status, tumor size and stage and is given in current guidelines.(25,26) Options include:(21)

- surgery (breast-preserving or mastectomy, sentinel lymph node biopsy, axillary dissection)
- radiation
- endocrinological therapy with hormone antagonists
- anti-Her2/neu therapy (trastuzumab, pertuzumab, lapatinib, trastuzumab emtansine)
- chemotherapy (anthracyclines, taxanes, cyclophosphamide, bendamustine, fluorouracil, gemcitabine, others)
- other targeted therapies (bevacizumab, everolimus, others)
- modulation of bone metabolism

While in the past surgery has been considered the primary treatment option for breast cancer, systemic therapy gained more relevance in recent years.(27,28) Depending on administration either before or after surgery it is distinguished between neoadjuvant and adjuvant therapy.(29,30) The aim of adjuvant therapy is the destruction or inhibition of micrometastases which may be present even after surgery and lead to future reoccurrence of the disease.(31) Neoadjuvant systemic therapy, which is also referred to as primary

systemic therapy or preoperative chemotherapy, may also enable surgery in formerly inoperable cases and increases the rate of breast conserving surgery. It also renders the possibility to monitor treatment response.(27,28)

Cardiotoxicity

1.2.5 Definition and Forms

The National Institute of Cancer defines cardiotoxicity as “toxicity that affects the heart”.(32) This simple definition basically covers all types of cardiovascular reactions in response to any cancer therapy. There are different types of cardiovascular complications that can be caused by anti-neoplastic therapy.(33,34) According to the European Society of Cardiology (ESC) nine different categories can be distinguished:(34)

- Myocardial dysfunction and heart failure
- Coronary artery disease
- Valvular disease
- Arrhythmias
- Arterial hypertension
- Thromboembolic disease
- Peripheral vascular disease and stroke
- Pulmonary hypertension
- Pericardial complications

However, in many cases the term cardiotoxicity more specifically refers to the impairment of left ventricular function and heart failure.(34) This thesis focuses on cardiotoxicity as chemotherapy-induced cardiomyopathy.

There is no common definition stating at which point a decline in left ventricular function is considered as cardiotoxicity.(35) The Cardiac Review and Evaluation Committee that supervised seven phase-II and III trastuzumab trials defined cardiotoxicity as a decline of the left ventricular ejection fraction (LVEF) of ≥5% to a final ejection fraction of <55% with symptoms of congestive HF or as an asymptomatic decline of LVEF ≥10% to a final ejection fraction of <55% detected by multigated acquisition (MUGA) scan or echocardiogram.(35) The American Society of Echocardiography (ASE) and the European Association of Cardiovascular Imaging (EACVI) defined a LVEF decline of ≥10% to a final LVEF of < 53% to be the limit for cardiotoxicity, determined by either echocardiography, MUGA scan or cardiac magnetic resonance imaging (MRI).(36) Apart from these two, more definitions and limits have been used by different authors.(35) For example, in the HERA trial which assessed trastuzumab-related cardiac adverse events a significant LVEF drop is considered as decrease of at least 10 absolute percentage points to

a value below 50% while the ESC mentions a LVEF decline of more than 10% to below the lower limit of normal.(34,37)

1.2.6 Classifications of Cardiotoxicity

Regarding the pathophysiology of cardiotoxicity, it is generally distinguished between type I and type II cardiotoxicity (see **table 4**).

Type I cardiotoxicity is typically caused by anthracyclines and the analogue mitoxantrone.(38) It is considered to be dose-dependent and irreversible.(39) Its mechanism is thought to be via myocyte cell death, and structural changes such as vacuole formation, myofibril disarray and necrosis have been described.(38)

Contrary, type II cardiotoxicity is considered being reversible because it is caused by myocyte dysfunction without cell death.(39) It is not dose-dependent. Typically causing agents are targeted therapies such as trastuzumab.(39,40) It has generally been described to be without ultrastructural changes.(40)

Cardiotoxicity	**Type I**	**Type II**
Characteristic agent	Doxorubicin	Trastuzumab
Clinical course/ response to therapy (ACE-inhibitors, beta blockers)	May stabilize, underlying damage appears to be permanent and irreversible	High likelihood of recovery
Dose effects	Cumulative, dose-related	Not dose-related
Effects of rechallenge	High probability of recurrent dysfunction that is progressive	Relative safety of rechallenge
Ultrastructure	Vacuoles, myofibrillar disarray and dropout, necrosis	No apparent ultrastructural abnormalities

Table 4: Characteristics of type I and II cardiotoxicity. Modified and shortened after (36)

Lately, this concept has been up to discussion. On the one hand it has been questioned whether trastuzumab-induced cardiotoxicity really is reversible, considering data suggesting that many patients continue to take cardiac medication and have persistent cardiac dysfunction shown by reduced LVEF during follow-up.(41) On the other hand

Cardinale et al. showed that early detection of anthracycline-induced cardiotoxicity with prompt initiation of pharmaceutic therapy can lead to an improvement of cardiac function in the majority of cases, despite it being considered irreversible.(42) Also ultrastructural changes in rat and mice cardiomyocytes could be demonstrated in type II cardiotoxicity, contradicting this commonly used classification.(43,44)

The ASE and the EACVI also classified cardiotoxicity as symptomatic or asymptomatic and according to its reversibility. A return to a value within 5 percentage points of baseline is considered reversible, a value that has improved by at least 10 percentage points from the nadir but remains more than 5 percentage points below baseline as partially reversible and a value that has improved by less than 10 percentage points from the nadir and remains more than 5 percentage points below baseline as irreversible.(36)

1.2.7 Further Drugs associated with Cardiotoxicity

As mentioned above cardiotoxic effects can be manifold. For the sake of completeness, a brief overview of the most important cardiotoxicity-inducing drugs is given in **table 5**.

Cardiac response	Drug	Incidence
Contractile dysfunction or heart failure	Anthracyclines	Cumulative, dose-related
	Cyclophosphamide	Rare
	Cisplatin	Rare
	Trastuzumab	Variable
	Lapatinib	
	Bevacizumab	Low
	Sunitinib	Low
	Sorafenib	Rare
	Imatinib	Rare
Arterial hypertension	All angiogenesis inhibitors	Moderate, dose-dependent
Myocardial ischemia	Pyrimidine analogues	Moderate
Thromboembolism	Cisplatin	Moderate
	All angiogenesis inhibitors	Moderate
Arrhythmia or QT prolongation	Arsenic trioxide	Moderate
	Lapatinib	Rare
	Sunitinib	Rare
	Nolitinib	Rare
	Dasatinib	Rare

Table 5: Most important cardiac side effects and causing agents. Modified and shortened after (39)

1.3 HER2/neu

1.3.1 Protein

ErbB2 is an 185,000 Dalton protein that is part of the epidermal growth factor receptor (EGFR) family. This family consists of four different receptors called EGFR (also called ErbB1 or HER1), ErbB2 (HER2 or neu), ErbB3 (HER3) and ErbB4 (HER4). Except for ErbB3 they all act as tyrosine kinases. They consist of an extracellular ligand-binding domain, a transmembrane domain and a cytoplasmic domain with the tyrosine kinase.(45)
All proteins of this family play an important role in the signal transduction cascade for cell proliferation, survival, adhesion, migration and differentiation of cells and therefore must be present in order to have normal cell functioning. However, in case of abnormal function they lead to the development and growth of tumor cells.(46)

1.3.2 Gene

The gene encoding for the ErbB2 protein can be found on band q21 of chromosome 17.(7)
The different but equivalent names ErbB2, neu, and HER2 for the gene and protein derive from the fact that different groups of scientists independently discovered these genes which were later shown to be the same.(7)

1.3.3 Signaling and Pathways

Since the different receptors of the EGFR family work together in signaling, it is not possible to describe only the ErbB2 receptor. Thus, a general explanation how the ErbB receptors contribute to signaling is given.
The tyrosine kinases of the receptors are inactive while the receptors are in their isolated form. They are activated by ligands binding on the extracellular domain, leading to the formation of hetero- or homodimers and hereby inducing tyrosine kinase activity at the cytoplasmic domain.(46) Ligands can be divided into three groups:(45)

1. The first group consists of EGF, transforming growth factor alpha (TGF-alpha) and amphiregulin (AR). These three specifically bind to EGFR.
2. The second group contains betacellulin (BTC), heparin-binding growth factor (HB-EGF) and epiregulin (EPR). These can bind to EGFR or ErbB4.
3. The third group includes the neuregulins (NRGs) and can be further divided depending on whether the ligands can bind to ErbB3 and ErbB4 (NRG-1 and NRG-2) or only to ErbB4 (NRG-3 and NRG-4).

All these ligands are growth factors belonging to the EGF (epidermal growth factor)-family and can be produced either by the same cells expressing the receptors (autocrine secretion) or by surrounding cells (paracrine secretion).(45,47)
There is no ligand known for ErbB2, however, it is the preferred partner for the other ErbB receptors to form heterodimers.(47)
Following the receptor activation is the phosphorylation of specific tyrosine residues within the cytoplasmic tail, which then serve as docking site for cytoplasmic enzymes and adapter proteins. The following dissociation of these signaling complexes releases activated effector and adaptor proteins into the cytoplasm which leads to activation of intracellular signaling pathways.(45,46)

The signal is terminated mainly through endocytosis of the receptor-ligand complex and later degradation or recycling of the resulting endosomes.(46)

1.3.4 Importance of ErbB2 in Development and the Heart

ErbB receptors are found in a variety of tissues. They are linked to early embryonic development and the renewal of stem cells in normal tissues like skin, liver or gut.(45) ErbB2 has been identified in the nervous system, developing bone, muscle, skin, heart, lungs and intestinal epithelium of human fetuses.(48)
ErbB2 seems to play a major role during cardiac development. Knockout mice without the ErbB2 gene die before birth which is probably due to trabeculae malformation of the heart.(49) But also in adults, ErbB2 plays an important role in adaptation and maybe also regeneration of the heart. Cardiac endothelial cells secrete NRG, which then binds to ErbB4 in myocardial tissue. ErbB4 then undergoes a conformation switch from closed to open conformation and forms ErbB4/ErbB4 homodimers, or ErbB4/ErbB2 heterodimers. Subsequent activation of tyrosine kinase activity and transphosphorylation of the ErbB cytoplasmic signaling tails leads to activation of cardioprotective signaling cascades. Although NRG-1 signaling is mainly associated with ErbB4, ErbB3 also seems to take part in these processes.(50)
This NRG-1/ErbB signaling axis is activated in heart failure and at least during the early stages compensates for the pathological changes leading to progression of cardiac dysfunction.(50)

1.3.5 ErbB2 in Breast Cancer

30 years ago, Slamon et al. showed that the ErbB2 gene is amplified in up to 30% of breast cancer cases and that this amplification is associated with a negative effect on survival rates and time to relapse.(7)

ErbB2 amplification plays a critical role in tumor growth. An important route is the formation of oncogenic ErbB2/ErbB3 complexes. Amplification of the ErbB2 gene leads to an uncontrolled and partially ligand-independent binding of the ErbB2 receptor to ErbB3. This results in the activation of the PI3K/Akt pathway and consequently in intense cell growth and proliferation.(50)

ErbB2 plays an important role not only in the pathogenesis of breast cancer but is also associated with other types of malignancies such as ovarian, gastric and bladder cancer.(50)

1.4 Trastuzumab

1.4.1 General Information and Clinical Use

Trastuzumab (brand name Herceptin®, Roche) is a humanized monoclonal antibody directed against the ErbB2 receptor.(51) It consists of two antigen-specific sites that bind to the extracellular domain of the HER2 receptor and the remainder of the antibody, which is human IgG with a conserved Fc portion.(52) The Fc region contains binding sites for Fcγ receptors present on immune cells, platelets, hepatocytes and endothelial cells.(53) By binding to the HER2 receptor trastuzumab inhibits the activation of its intracellular tyrosine kinase.(52,54)

In a phase-III trial Slamon et al. showed that trastuzumab leads to significantly longer time to disease progression, a higher rate of response, a longer duration of response and improved overall survival.(9) Trastuzumab was first approved by the Food and Drug Administration (FDA) for clinical use in HER2-positive metastatic breast cancer in the United States in 1998 and in 2000 also by the European Medicines Agency (EMA) for use in the European Union.(51) Today it is also approved for early Her2-positive breast cancer and metastatic gastric cancer.(55)

In breast cancer patients, Trastuzumab can be administered intravenously in three-week intervals or weekly. In the weekly regimen, the loading dose is 4 mg/kg body weight and the maintenance dose 2 mg/kg body weight, while in the three-week regimen the loading dose is 8 mg/kg body weight and the maintenance dose is 6 mg/kg body weight. If administered intravenously Trastuzumab should be administered over 30-90 minutes per infusion cycle.(55) Trastuzumab can also be administered subcutaneously. The HannaH study demonstrated the non-inferiority of subcutaneous administration and a similar safety profile compared to intravenous administration.(56) In this regimen, a dose of 600 mg is administered subcutaneously over 2-5 minutes every three weeks.(55) In 2017, the PrefHer study showed that a great majority of patients preferred subcutaneous administration.(57)
In early breast cancer patients, individual studies have shown effectiveness of a nine-week period of treatment with Trastuzumab comparable with treatment for a year.(58) However, currently 12-months is considered the optimum duration of treatment in early breast cancer, which is also reflected by guidelines.(55,59) In metastatic breast cancer patients treatment is continued until disease progression.(55)

1.4.2 Mechanism of Action

Several mechanisms how trastuzumab works in fighting against HER2 positive cancer have been described.(52,60,61)

Interference with the ErbB2 receptor

A crucial point seems to be the interference with the ErbB2 receptor. Blocking of ErbB2 dimerization leads to subsequent inhibition of the signaling pathways mentioned above. This is thought to lead to apoptosis and arrest of proliferation in cancer cells.(60) Apart from this the destruction of the HER2 receptor via endocytosis, which is thought to be caused by the binding of trastuzumab, may lead to the reduction of relative cell surface expression, in other words HER2 downregulation, and thus to reduced signaling.(60,61)

Antibody-dependent cellular cytotoxicity

Another mechanism might be the activation of antibody-dependent cellular cytotoxicity (ADCC). Natural killer cells expressing the Fcγ receptor are activated and can then bind to the Fc domain of trastuzumab. This leads to the lysis of tumor cells which are bound to trastuzumab.(60) This theory has been supported by findings of Clynes et al. who showed that in mice lacking the Fc receptor only 29% tumor growth inhibition was observed compared to 96% in mice with the Fc receptor.(62)

HER2 cleavage

Additionally, the inhibition of HER2 cleavage seems to play a role. The HER2 receptor undergoes proteolytic cleavage when it is over-expressed. This leads to shedding of the extracellular domain which is then released into the blood, and leaves the membrane-bound fragment called p95.(60,63) This fragment is phosphorylated and exhibits kinase activity, enabling potential signaling.(63) Trastuzumab has been shown to inhibit this shedding process.(64) Decreased serum levels of the extracellular domain have been associated with a more favorable treatment outcome, supporting this theory.(60,65)

Further mechanisms

Also, a connection between HER2 overexpression and increased angiogenesis has been demonstrated by Yen et al.(66) Studies with mice have shown the normalization of vasculature due to treatment with trastuzumab, indicating a possible inhibition of angiogenesis provoked by trastuzumab.(60)

Furthermore, trastuzumab seems to affect DNA repair. It has been shown that cells with HER2 overexpression show up to 44% less DNA repair action after cisplatin or radiotherapy when trastuzumab is present.(61)

1.4.3 Adverse Events

The most severe side effects of trastuzumab therapy include cardiac dysfunction, infusion reactions, hematologic toxicity with neutropenia, infections and pulmonary toxicity.(55,67) Other common side effects include, amongst others, arthralgia, asthenia, headache, emesis and diarrhea.(57,67) Excluding injection site reactions, the rates of adverse events are similar in intravenous and subcutaneous administration.(57)

1.5 Trastuzumab-induced Cardiotoxicity

1.5.1 Epidemiology

In 2001 Slamon et al published the results of a phase 3 trial which showed the extent of cardiotoxic effects caused by trastuzumab. Different from expectations based on results from earlier studies cardiac dysfunction was present in up to 27% of cases when patients were treated with anthracyclines, cyclophosphamide and trastuzumab. In patients receiving paclitaxel and trastuzumab there was a 13% incidence. In patients who received an anthracycline and cyclophosphamide alone cardiac dysfunction was present in only 8%, in patients receiving paclitaxel alone it was apparent in 1%. Comparing all cases the incidence of cardiac dysfunction according to New York Heart Association (NYHA) class III and IV was also highest in patients receiving anthracyclines, cyclophosphamide and trastuzumab (16%), while it was 3% in patients receiving anthracyclines and cyclophosphamide alone, 2% in patients with paclitaxel and trastuzumab and 1% in those treated with paclitaxel alone.(9)

As a result, the administration regimens were changed to allow for a longer interval between chemotherapy and trastuzumab administration and cardiac monitoring during trastuzumab therapy was established.(34) Also, patients with significant cardiovascular history were excluded from studies and there was a stricter protocol for the use of trastuzumab based on LVEF results.(68)

In a meta-analysis of randomized and cohort studies including 29,000 women, which was published in 2015 severe cardiotoxicity occurred in 3% of overall breast cancer patients treated with trastuzumab. Severe cardiotoxicity was defined as congestive heart failure, myocardial infarction, cardiac dysrhythmia, LVEF reduction or other cardiac toxicity grade III/IV according to the NCI Common Toxicity Criteria for Adverse Events or NYHA classification.(59)

Overall the incidence of left ventricular dysfunction associated with trastuzumab therapy differs among studies and has been variably described to be between 1.7 - 20.1%.(34)

1.5.2 Limitation for Cancer Therapy

According to the official Herceptin medical information trastuzumab therapy should be paused in case of LVEF decrease of both 10 or more percentage points below the baseline value and to a value below 50%. Cardiac function should be reevaluated after three weeks

and discontinuation of trastuzumab therapy should be seriously considered if there is no improvement or even worsening of LVEF or symptomatic heart failure.(69)

1.5.3 Risk factors

Established risk factors for the development of trastuzumab-induced cardiotoxicity are: (34,59,70–73)

- Advanced age
- Smoking
- Arterial hypertension
- Dyslipidemia
- BMI > 25 kg/m²
- Diabetes
- Postmenopausal status
- Previous or simultaneous anthracycline therapy
- Positive history for cardiac disease or coronary artery disease
- Borderline LVEF or previous LV dysfunction

Other possible risk factors might be genetic background and the patient's immune status.(74)

1.5.4 Mechanism of Cardiotoxicity

Although the exact mechanism of trastuzumab-induced cardiotoxicity is still not completely understood, several possible mechanisms have been proposed.(61) These are summarized in **table 6**.

Proposed mechanisms for trastuzumab-induced cardiotoxicity
Inhibition of ErbB2 signaling pathways and ErbB2 downregulation
ADCC (immune-mediated)
Mitochondrial dysfunction
Changes in gene expression
Structural damage to myofibrils
Inhibition of autophagy
Effects on myocardial endothelium
Drug interactions (e.g. with anthracyclines)

Table 6: Mechanisms of trastuzumab-induced cardiotoxicity. (33,71,74–77)

Inhibition of ErbB2 signaling pathways

A central mechanism seems to be the inhibition of ErbB2 signaling pathways.(74) As described above, these play a major role in metabolism, growth, protection and survival of the cardiomyocyte.(77) The heterodimerization of ErbB2 and ErbB4 receptors, induced by neuregulin-1, activates signaling pathways important for cell survival. By blocking these pathways trastuzumab may downregulate survival signals and lead to myocyte injury and loss of repair mechanisms when the cell is exposed to stress.(61) However, lapatinib, a dual tyrosine kinase inhibitor of EGFR and ErbB2, seems to be less cardiotoxic despite also interfering with ErbB2 signaling.(74,78,79) This indicates that there might be a mechanism not related to ErbB2 inhibition in general but related more specifically to trastuzumab.(33)

Immune mediation

Also, an immune-mediated effect on cardiomyocytes is discussed. Since trastuzumab has been shown to stimulate ADCC against HER2 positive tumor cells, it seems reasonable that there might also be a reaction directed against cardiomyocytes.(74) However,

pertuzumab, which also is a monoclonal antibody blocking ErbB2 dimerization, seems to be less cardiotoxic.(74,78)

Mitochondrial dysfunction

Also playing a role in cardiotoxicity might be effects of oxidative and nitrosative stress and mitochondrial dysfunction caused by ErbB2 inhibition.(44,53,61). It has been proposed that trastuzumab affects the regulation of mitochondrial integrity through BCL-X proteins after binding to ErbB2, thus leading to ATP depletion and contractile dysfunction without causing structural defects of the myocytes.(33,74) Grazette et al. have shown that inhibition of ErbB2 in rat cardiomyocytes leads to an increase in expression of the pro-apoptotic protein Bcl-xS and a decrease in expression of the anti-apoptotic protein Bcl-xL. This change in Bcl-xS/Bcl-xL-ratio leads to increased protein levels of BAX and increased oligomerization of BAX. This is associated with an increase in cytosolic cytochrome c, another pro-apoptotic protein, and subsequent caspase activation. Due to these alterations mitochondrial dysfunction, a loss of mitochondrial membrane potential, a decline in ATP levels and a loss of redox capacity could be observed. However, despite the activation of mitochondrial apoptotic signaling only a modest increase of apoptosis could be measured. The authors suggest that consequences of incomplete progression to apoptosis, like mitochondrial dysfunction, may lead to functionally impaired cardiomyocytes and therefore play a role in cardiotoxicity.(80)

It was also shown that treatment with trastuzumab increases myocardial oxidative and nitrosative stress in mice. An increase of caspase 3/7 activity was shown, indicating the activation of apoptotic pathways. However, similar to results of the study described above, DNA fragmentation as a sign of apoptosis in mice treated with trastuzumab did not show a statistically significant difference compared to the control group.(44)

Gene expression

It has also been shown that trastuzumab alters the expression of certain genes in an animal model. ElZarrad et al. identified 15 genes that were affected by trastuzumab. These genes were then divided into two groups. The first group included nine genes with involvement in cardiac contractility, adaptation to stress and hemodynamic pressure, DNA repair, proliferation, wound healing and mitochondrial function. In other words, all of them had direct effects on cardiac function. Eight of these genes were significantly decreased in mice

that have been treated with trastuzumab, one was increased. The second group consisted of six genes that modulate circadian rhythm and thereby influence heart rate, homeostasis, oxidative stress and mitochondrial function. Two of these genes showed a decrease, the other four an increase in expression.(44)
Changes in gene expression were also shown in human iPSC (induced pluripotent stem cell)-derived cardiomyocytes. Necela et al. showed a downregulation of expression of genes associated with ischemic preconditioning, cardiac dysfunction and metabolism.(75)

Further mechanisms

Contradicting the view that in trastuzumab-induced cardiotoxicity no histological changes can be observed in the myocyte, ultrastructural damage of heart tissue in mice has been shown. These changes observed with electron microscopy include stretched myofibers with reduced thickness and a decreased number of mitochondria.(44)

A recent study has shown another possible mechanism of trastuzumab-induced cardiotoxicity via inhibition of autophagy. It has been proposed that by interfering with ErbB2 signaling trastuzumab activates Erk, which leads to an upregulation of the mTor-Ulk1 pathway. This then leads to inhibition of autophagy in cardiomyocytes. This consequently may prevent the efficient degradation of damaged cytoplasmic materials.(76) It has previously been shown that inhibition of autophagy causes an accumulation of ROS.(76,81) Contrary to trastuzumab, pertuzumab did not have a downregulating effect on autophagy.(76)

Apart from effects on the myocyte, ErbB2 signaling pathways may also influence the cardiac endothelium. ErbB2 is present in endothelial cells where it seems to play a role in angiogenesis and autocrine and paracrine regulation of the heart. Studies have shown that deletion of neuregulin in endothelium causes increased susceptibility of myocytes towards ischemia-reperfusion injury.(77)

The clinical important interaction between anthracyclines and trastuzumab will be explained in the following chapter.

1.6 Anthracycline-induced Cardiotoxicity

Since previous or concomitant anthracycline therapy is a major risk factor in trastuzumab-induced cardiotoxicity, basics of this substance group will also be briefly explained in this thesis.

1.6.1 General Information and Mechanism of Action

Anthracyclines are antibiotic drugs derived from Streptomyces bacteria which are used in cancer therapy because of their cytotoxic effects.(82) They were introduced in the 1960s and since then are an important part of chemotherapy for different types of cancer such as lymphoma, breast cancer and small cell lung cancer.(83) Of all breast cancer patients, 32% are treated with anthracyclines.(84) The most important anthracycline drugs are doxorubicin, daunorubicin, idarubicin and epirubicin.(84) Chemically anthracyclines consist of an aglycone with a tetracyclic ring structure and a sugar called daunosamine, which is attached to the tetracyclic ring by a glyosidic bond.(85)

In summary, anthracyclines promote DNA damage.(83) There are several mechanisms contributing to the anti-neoplastic effect of anthracyclines. An intercalation of the planar ring structure between adjacent DNA pairs leads to inhibition of protein synthesis and DNA replication. Due to the formation of reactive oxygen species DNA damage and lipid peroxidation take place. Additionally, DNA damage is also caused by inhibition of the topoisomerase-2 enzyme. DNA cross-linking, binding and alkylation lead to blockage of DNA replication and/or transcription. Apart from these two mechanisms mentioned, anthracyclines also have a direct effect on membranes, leading to the disruption of the bilayer membrane structure.(85)

1.6.2 Epidemiology of Anthracycline-induced Cardiotoxicity

Anthracycline-induced cardiotoxicity is highly dose dependent. With epirubicin doses >900 mg/m2 it lies between 0.9–11.4% and in case of doxorubicin its incidence lies between:(34)

- 3-5% for doses up to 400 mg/m²
- 7-26% for doses of 550 mg/m²
- 18-48% with doses of 700 mg/m².

1.6.3 Mechanism of anthracycline-induced Cardiotoxicity

The process of anthracycline-induced cardiotoxicity is thought to be multifactorial and the exact mechanism remains unknown (84). However, reactive oxygen species (ROS) and topoisomerase-2β seem to play a major role.(83) Mele et al. have tried to unite the main hypotheses currently available to explain anthracycline cardiotoxicity at a cellular level.(85) This is summarized in **figure 2**.

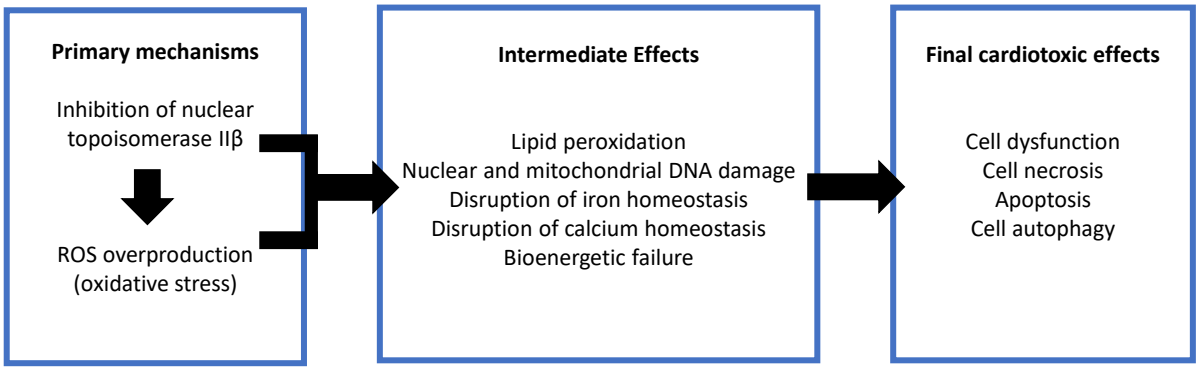

Figure 2: Main hypotheses for anthracycline-induced cardiotoxicity. Modified after (85)

Reactive oxygen species (ROS)

Until now, the most accepted theory was that anthracyclines interfered with redox cycling, leading to production of reactive oxygen species (ROS) and thereby causing DNA damage.(84) ROS formation might be induced by anthracyclines via an enzymatic pathway, aglycone redox reaction and by formation of iron complexes.(86) These free radicals then target DNA molecules, proteins and cellular membranes. DNA damage and lipid peroxidation subsequently lead to cell death and organ damage.(85) Since cardiomyocytes do not contain high levels of free radical scavengers like catalase and glutathione peroxidase they are even more susceptible to damage caused by this process.(86) This theory seems to be supported by observations which show a reduction of cardiotoxicity rates in patients treated with the iron chelator dexrazoxane concomitantly to anthracycline therapy.(83)

Topoisomerase-2β

Recently, the role of topoisomerase (top) 2 has been discussed to play a major role in causing cardiotoxicity.(84) DNA topoisomerases cause temporary single or double-stranded breaks to regulate the topological changes during DNA replication, transcription,

recombination and chromatin remodeling. There are two isoenzymes in humans called topoisomerase 2α and 2β. Top 2α is present in proliferating malignant and non-malignant cells, whereas top 2β is present in quiescent cells such as adult cardiomyocytes. Doxorubicin binds with DNA and topoisomerase 2 to form a complex which causes double-stranded DNA breaks. In cancer cells expressing top 2α this induces apoptosis, which is the intended process.(84) However, doxorubicin interferes with both top 2α and 2β.(83) In cells expressing top 2β, doxorubicin also leads to formation of a doxorubicin-top 2β-DNA-complex. Like with the intended mechanism in anti-neoplastic therapy this complex causes DNA breaks and subsequently leads to increased apoptosis and thereby cardiac cell death.(83,84) This theory is supported by findings of animal studies which have shown that the absence of top 2β in top 2β-knock out-mice is a protective factor against doxorubicin-induced cardiotoxicity.(87,88)

Further mechanisms

Also, other mechanisms have been discussed to play a role in anthracycline toxicity. It has been proposed that anthracyclines might damage major structural proteins such as titin, which is a myofilament forming protein, or dystrophin, a sarcomere protein. This may consequently lead to systolic or diastolic cardiac dysfunction and dilated cardiomyopathy.(86)

Another theory is apoptosis due to upregulation of death receptors. It was found that in iPSC-derived cardiomyocytes treatment with doxorubicin induced upregulation of the death receptors TNFR1, Fas, DR4 and DR5. This was detectable both at mRNA and protein levels. These changes have been shown also after treatment with daunorubicin, idarubicin and epirubicin, however, they could not be demonstrated at all or not to the same extent after treatment with sunitinib, Herceptin, Fluorouracil (5-FU) or Taxol. An increase in apoptotic cell population following treatment with doxorubicin could be shown by increased DNA fragmentation.(89)

Extracellular matrix remodeling and loss of iron and calcium homeostasis due to alcohol metabolites also seem to be important factors. Since not all patients react to comparable doses of anthracycline therapy in the same way, a genetic predisposition for the development has also been discussed.(85)

1.6.4 Interaction between Anthracyclines and Trastuzumab

One risk factor for the development of trastuzumab-induced cardiotoxicity is previous or concomitant anthracycline therapy.(34,70) In a small study Korte et al have found that myocardial HER2 was over-expressed in 50% of patients having received anthracyclines in the past three weeks. This is thought to be a response to cardiac stress caused by anthracyclines.(90) It has been proposed that trastuzumab might consequently lead to cardiac dysfunction by aggravating pre-existing subclinical cardiac damage caused by anthracyclines or via a multiple-hit hypothesis. In this case cardiac stress after anthracycline therapy is considered the first hit while the inhibition of HER2 signaling caused by trastuzumab is thought to be the second hit.(90)

1.7 Diagnosis of Cardiotoxicity

1.7.1 Imaging Techniques

1.7.1.1 Echocardiography

Echocardiography is the most commonly used method for monitoring cardiac function and is recommended by the ESC before, during and after chemotherapy.(34,91)

LVEF

The most commonly accepted parameter of cardiac function is the LVEF, which has also been shown to be an independent predictor of mortality from cardiovascular events and anthracycline-induced cardiotoxicity.(42,91) Advantages of echocardiographic examination are its high availability, reproducibility and noninvasive nature.(91) Furthermore, it offers the assessment of both function and cardiac structure.(34) Limitations however include inter-observer variability, differences in image quality, assumption of LV geometry, the dependence on (pre- and after-) load and the examiner's expertise.(34,91) Studies have shown a detection rate of 98% with close echocardiographic monitoring for 12 months in cases of anthracycline-induced cardiotoxicity.(42) However, the correlation between cardiac biopsy grades and LVEF is poor in patients receiving doxorubicin.(92) This suggests that echocardiography might be a rather unsensitive tool in early detection of cardiotoxicity due to the fact that a decrease in LVEF occurs only after cardiac compensatory mechanisms have failed.(91,93) It has also been shown that in many cases diastolic dysfunction might precede LVEF decrease.(42) However, at this point there is no recommendation to stop treatment based on this result.(34) In a study with HER2-positive breast cancer patients receiving anthracyclines, taxanes, and trastuzumab neither the LVEF measured after completion of anthracycline therapy nor the change between LVEF at baseline and after completion of anthracycline therapy could predict future cardiotoxicity.(94)

In its 2016 position paper the ESC recommends echocardiography as method of choice for the detection of cardiac dysfunction before, during and after chemotherapy. Three-dimensional echocardiography is the preferred method of examination. In cases where this technique is not available the two-dimensional biplane Simpson method is recommended.(34)

Myocardial Strain

A newer method which might be beneficial in detecting cardiotoxicity at an early stage is strain echocardiography.(34) This examination, also called deformation imaging, can be used to evaluate the regional myocardial mechanical function in an objective manner.(95) It has been demonstrated that subclinical changes can be detected before a decrease in LVEF takes place.(91) The global systolic longitudinal myocardial strain seems to be a reliable parameter in predicting future cardiotoxicity.(34) In the above-mentioned study Sawaya et al. demonstrated that longitudinal strain measured after completion of anthracycline therapy was predictive of future cardiotoxicity. A value of <19% was chosen to detect high-risk patients. No predictive value could be found for radial or circumferential strain.(94) However, several limitations prevent the wide-spread use of this method. Among these are an offline and time-consuming data analysis, dependency on the acoustic window and different machines and software programs resulting in low comparability of results. In addition to that no long-term data are available at this moment.(91)

1.7.1.2 MUGA-Scan

Another well-established examination technique to monitor and evaluate cardiac function is the multiple-gated acquisition (MUGA) scan, also known as radionuclide angiography.(34) Major advantages of this method include its good accuracy, reproducibility and limited inter-observer variability.(34,91) However, it exposes the patient to radiation, especially if used repeatedly during monitoring, and in addition to that gives only limited information on hemodynamics, cardiac structure and diastolic function.(34,91)

1.7.1.3 Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance is considered the gold standard in evaluating systolic and diastolic function, cardiac volumes and cardiac mass.(91) It helps in determining causes of left ventricular dysfunction and can also detect cardiac fibrosis. Furthermore, it is highly accurate and reproducible and does not expose the patient to radiation. It is limited though by lower availability, higher costs and also might not be tolerated by all patients due to claustrophobia or other reasons.(34,91)

1.7.2 Cardiac Biomarkers

Several biomarkers have been studied in their ability to detect cardiotoxicity at an early stage or even predict its future appearance.

1.7.2.1 Troponin

In studies with pediatric patients receiving high dose chemotherapy containing anthracyclines for acute lymphoblastic leukemia, an elevation of troponin T levels is associated with both a higher risk for the development of cardiotoxicity and worse outcomes.(96,97) In adults, troponin I elevation is associated with a future decrease in LVEF in patients receiving high dose chemotherapy.(98) Cardinale et al. demonstrated the predictive value of troponin I. In their study, they identified three different groups regarding the pattern of troponin I elevation after high dose chemotherapy by measuring its values soon after chemotherapy (early troponin) and one month after its completion (late troponin). In patients with negative early and late troponin values no significant LVEF reduction and only a 1% incidence of cardiac events during follow-up could be observed. In patients with positive early troponin and negative late troponin a 37% incidence of cardiac events was observed compared to 84% in patients with both early and late positive troponin values. The authors determined a 84% positive predictive value and a 99% negative predictive value for troponin I regarding the occurrence of cardiac events during follow-up.(99) In patients receiving trastuzumab it has also been shown that an elevation of troponin I at baseline or during therapy is associated with a higher incidence of cardiotoxicity and a lower rate of recovery despite heart failure therapy.(100)

1.7.2.2 BNP

The role of natriuretic peptides in defining high-risk patients in those receiving chemotherapy is not clearly established at the moment.(34) For the use of anthracyclines studies have shown that in patients receiving high-dose chemotherapy an elevation of BNP>100 pg/ml at baseline or at any point during therapy might be a risk factor for the development of cardiotoxicity.(101,102) Persistent elevations of NT-proBNP values 72 hours after the end of high-dose chemotherapy are also associated with the occurrence of cardiac dysfunction during the following 12 months.(103) However, not all studies have shown a clear predictive value of natriuretic peptides, which might be due to different

timing of measurement, different occurrence of cardiac risk factors in different study populations or differences in the assays used and their cut-off values.(104)

Some small studies also found an association between elevated NT-proBNP levels in patients receiving trastuzumab and cardiotoxicity.(105) However, in several other studies no significant association between elevated values of NT-proBNP and the development of cardiotoxicity in patients treated with trastuzumab could be demonstrated.(94,106)

1.7.2.3 Further Biomarkers

Different biomarkers have been evaluated. Ky et al. evaluated the association between early increase of biomarker levels and the risk of subsequent cardiotoxicity in patients receiving anthracyclines, taxanes, and trastuzumab. Biomarkers they tested were ultrasensitive troponin I (TnI), high-sensitivity C-reactive protein (CRP), N-terminal pro–B-type natriuretic peptide (NT-proBNP), growth differentiation factor (GDF)-15, myeloperoxidase (MPO), placental growth factor (PlGF), soluble fms-like tyrosine kinase receptor (sFlt)-1, and galectin (gal)-3. No association of baseline levels with cardiotoxicity could be shown for any biomarker, although MPO showed marginal significance. Considering the interval change from baseline to visit two (at three months) an association with subsequent cardiotoxicity could be demonstrated for hs-TnI and MPO. Similar, but not statistically significant, results could be shown for GDF-15 and galectin-3 levels. No association could be demonstrated for NT-proBNP, CRP, placental growth factor, or soluble fms-like tyrosine kinase receptors.(107) In a further study, the authors evaluated the association between longitudinal changes of these biomarkers and the risk for cardiotoxicity. An association between elevated levels and increased risk for concurrent cardiotoxicity at this visit could be established for MPO, PlGF and GDF-15. High-sensitive CRP showed only marginal significance in this setting. When considering elevated biomarker levels and increased risk for cardiotoxicity at the subsequent visit similar results were found for MPO and GDF-15. PlGF showed comparable, however not statistically significant results, whereas gal-3 did show a significant association in this setting. No association between elevated biomarker levels and increased risk of cardiotoxicity could be demonstrated for hs-cTnI and NT-proBNP. For troponin, this was contrary to earlier results and might be due to a larger fraction of missing data or a different pattern compared to the other biomarkers in this cohort. Also, no association with cardiotoxicity could be found for sFlt-1.(108)

However, at this time neither clear indications nor the exact timing for measurement of the different biomarkers are known. Despite the positive results demonstrated there are still no recommendations on how to respond to subtle rises of these biomarkers. Another limitation is the variability depending on different assays, leading to lower comparability of results. (34)

1.8 Therapy

1.8.1 Prevention

No clear indications for cardioprotective therapy exist at this point of time. According to the current ESC position paper prophylactic cardioprotective medication might be considered in patients at high risk for cardiotoxicity or those who will receive a high cumulative anthracycline dose. For patients with low baseline risk no recommendation can be made so far.(34)

1.8.1.1 Current Studies

Several current studies have aimed at investigating potential preventive effects of heart failure therapy administered concomitantly with cancer therapy. Up to this point however, they have delivered inconclusive results for the effects of ACE inhibitors (ACEIs), beta blockers (BBs) and angiotensin II receptor blockers (ARBs).(10,109–112)

The OVERCOME trial showed a protective effect of the ACE-inhibitor enalapril and the beta blocker carvedilol on left ventricular systolic function in 90 patients receiving intensive chemotherapy containing anthracyclines for hematological malignancies or with autologous hematopoietic stem cell transplantation. While in the intervention group LVEF remained the same a decline of 3.1% measured by echocardiography or 3.4% measured by cardiac MRI could be observed in the control group. Also, less clinical events occurred in the intervention group compared to the control group.(110)

In the PRADA trial Gulati et al. could demonstrate a positive effect of the angiotensin II receptor blocker candesartan on LVEF decline in 130 patients with early breast cancer receiving adjuvant anthracycline therapy with or without trastuzumab and radiation. Overall LVEF decline was 0.8% in the candesartan group compared to 2.6% in the placebo group. No effect could be shown for the beta blocker metoprolol.(109)

The MANTICORE-101 Breast study evaluated the effects of the ACE-inhibitor perindopril and the beta blocker bisoprolol on left ventricular remodeling in 159 patients who received trastuzumab therapy for early breast cancer. It could be demonstrated that both perindopril an bisoprolol lead to lower LVEF decline measured by cardiac MRI and to fewer treatment interruptions caused by LV dysfunction. Mean absolute LVEF change was lower in the bisoprolol group than in patients receiving perindopril or placebo. However, for both drugs no effect on left ventricular remodeling measured by indexed left ventricular end diastolic volume (LVEDVi) could be observed.(10)

A cohort study with breast cancer patients receiving trastuzumab and/or anthracycline therapy found a favorable effect on cardiotoxicity rates in the patient group exposed to ACEIs or BBs.(111) A different study investigating the effect of the angiotensin II receptor blocker candesartan administered concomitantly to trastuzumab therapy in early breast cancer patients could not show a beneficial effect regarding LVEF reduction rates.(112) More studies are still ongoing. (113–115)

1.8.2 Heart Failure Therapy

Heart failure therapy according to current guidelines should be considered in patients with asymptomatic LVEF decline and should be initiated in patients with clinical heart failure.(34) Therapy options include but are not limited to ACEIs, ARBs, BBs, mineralocorticoid receptor antagonists, diuretics and further medication.(116)

Since studies have shown favorable results cardioprotective therapy with enalapril might be initiated in patients showing a troponin increase during high dose anthracycline therapy. (34,42)

2 Material und Methods

2.1 Study design and Scientific Question

The study we conducted for this thesis is a single-center retrospective study. It aims at giving an overview of the incidence of cardiotoxicity in the study cohort and at identifying possible connections between the deterioration of heart function during or shortly after trastuzumab therapy and pre-existing medical conditions and pharmaceutical co-therapy.
Primary objective is the comparison of echocardiographic indices (left ventricular function and left ventricular dimension) and cardiac biomarkers before, during and after trastuzumab treatment. Secondary objective is the comparison of pre-existing medical conditions and pharmaceutical co-therapy.

2.2 Study Population

2.2.1 Inclusion Criteria

In this study we included all female patients who received treatment with trastuzumab for breast cancer at the Department of Oncology at the Medical Clinic of the University of Graz (LKH Graz) during the years 2004 and 2016. All patients were included irrespective of pre-existing medical conditions and pharmaceutical co-therapy. Also, all patients were included irrespective of specific breast cancer identity and additional cancer treatment.

2.2.2 Exclusion Criteria

We excluded all cases were no definitive answer regarding the completion of therapy was available due to missing data or in which therapy was still ongoing at time of data collection. We also excluded patients who did not receive full therapy at the Department of Oncology in at the LKH Graz or who died during the treatment phase (not therapy related). Detailed reasons for exclusion can be reviewed in **figure 3**.

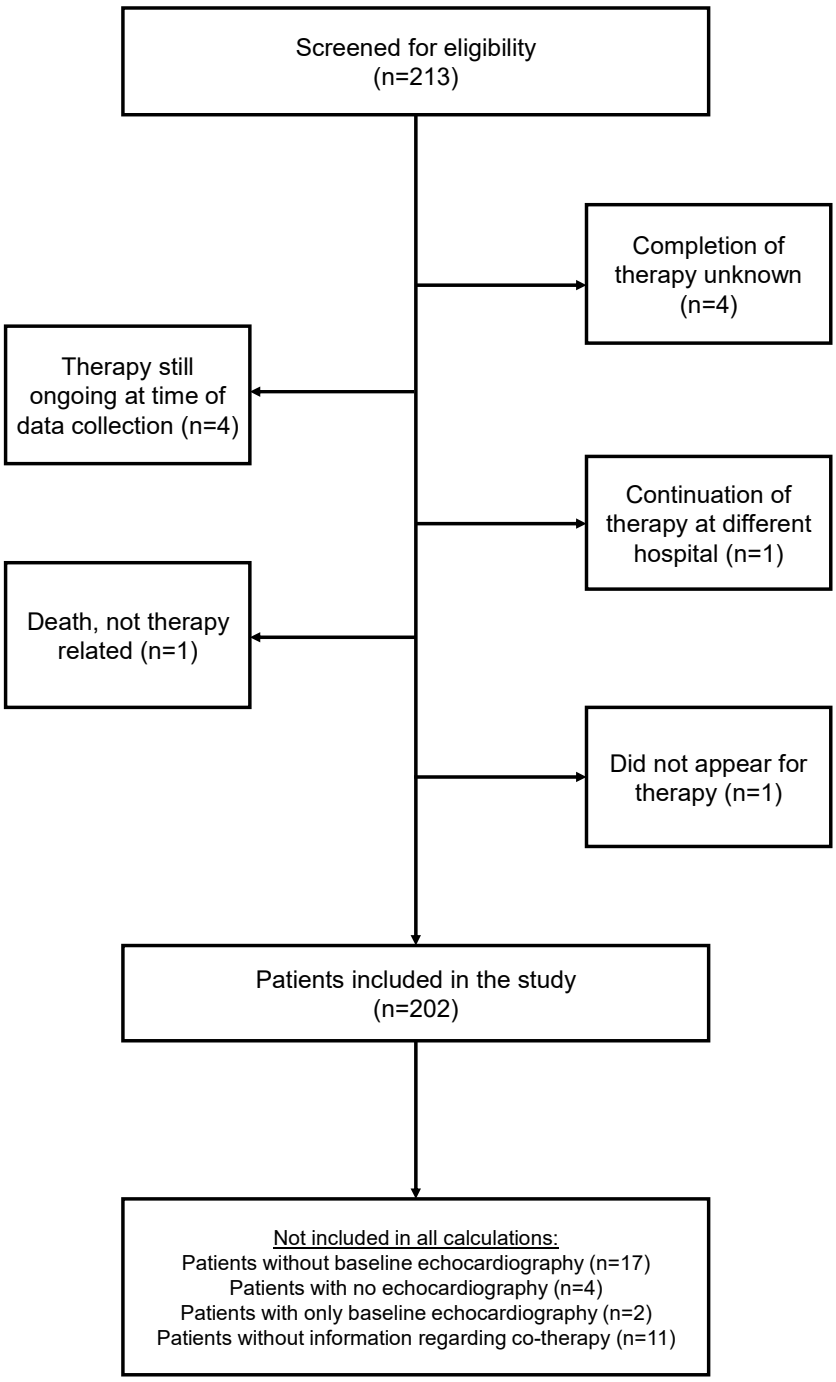

Figure 3: Inclusion and exclusion into the study.

2.2.3 Final Study Cohort

A total of 202 female patients was included in the final study. Patients without baseline echocardiography, with only the baseline echocardiography, with no echocardiography at all and patients without information regarding medical co-therapy were included in descriptive statistics but were excluded from certain analyses.

2.3 Data Collection

Approval was obtained from the ethics committee at the MUG. Clinical data of all patients who received trastuzumab therapy during the time frame stated above was automatically retrieved from the hospital information system MEDOCS and saved in excel files. This data included date of birth, specific information on tumor identity and cancer therapy. Also results of laboratory blood analysis and results from all echocardiographic examinations performed at the University Hospital of Graz were automatically retrieved from the information system MEDOCS and 4D echocardiography and saved in excel files. Additionally, medical records from the Department of Cardiology and Department of Oncology were retrieved from the information system MEDOCS and saved in pdf-format. Paper files of participating patients were searched for additional results of echocardiographic examinations performed outside the University Hospital and for existing pharmaceutical co-therapies as well as to confirm the integrity of automatically retrieved data. To ensure data security the original files were password protected and could only be accessed by authorized personal. The final excel data sheet (Microsoft Excel 2016) was created using pseudonyms.

2.4 Parameters

2.4.1 Demographics and Clinical Data

General data that was determined for each patient included age, weight, length, body mass index (BMI), Karnofksy performance score (KPS), tumor identity (localization, grade, stage, hormone receptor status, proliferation index) and additional cancer therapy (chemotherapy, radiation, hormonal therapy, additional antibodies). Data was retrieved from medical records and the MEDOCS information system, information regarding weight length and BMI were additionally derived from echocardiographic examination logs.

2.4.2 Discontinuation of Therapy

For each patient it was determined whether they received complete treatment or if therapy with trastuzumab was discontinued before completion. In this case the exact reason for discontinuation was documented as well as whether it was due to cardiotoxic effects or different motives.

2.4.3 Echocardiographic Indices

For the assessment of cardiac function all echocardiographies were considered, irrespective of whether they were performed at the University Hospital in Graz or externally. Cardiac function was evaluated using the following indices:

- Left ventricular ejection fraction (LVEF)
- Left ventricular function (LVF) category
- Left ventricular end-diastolic dimensions (LVEDD)
  - M-mode
  - 2D
  - Combined value
- Left ventricular (LV) size category

For all parameters two values were selected. The first value was the baseline value. As baseline we selected the echocardiography that was performed closest to or at the date of trastuzumab initiation, however, in any case no later than that day. The second value was selected from all echocardiographies that were available for a patient during an interval of two years after trastuzumab initiation date. This timeframe was selected to ensure comparability and to prevent other influential factors. The exact point of time of the second value differs among patients and may also vary for different parameters in each patient.

LVEF

For the LVEF the minimal value that was documented during the two-year interval was chosen as second value. If no numerical value was given for either baseline or 2-year LVEF value, but a general category description was available the value was determined accordingly. In these cases normal left ventricular function was set as EF = 54% according to Lang et al.(117) For borderline left ventricular function we arbitrarily determined the EF to be 53%. We then determined the difference between LVEF at baseline and the minimal value during the two-year interval. We also classified minimal LVEF according to the categories proposed by the ASE/EACI as described in **table 7**.

Category	LVEF %	LV diast. diameter (mm)
Normal range	54–74	38–52
Mildly abnormal	41–53	53–56
Moderately abnormal	30–40	57–61
Severely abnormal	<30	>61

Table 7: Ranges for EF and LV diastolic diameter derived from 2D echocardiography in female patients according to ASE/EACI. Modified after (117). diast.=diastolic

LVF Category

Left ventricular function was also described using the categories utilized at the University Hospital (LKH) in Graz. These are:

- Hyperdynamic systolic function
- Normal systolic function
- Borderline systolic function
- Mildly reduced systolic function
- Mildly-moderately reduced systolic function
- Moderately reduced systolic function
- Moderately-severely reduced systolic function
- Severely reduced systolic function

Both the baseline value and the poorest category during the two-year interval were selected and it was determined whether a change of categories took place or not.

LVEDD

For LVEDD we determined the baseline and highest value of both M-Mode and 2D examination during the two-year interval. In a second step we combined both values to form a LVEDD (combined) value. This was done to ensure optimum use of available records if one measurement was lacking and given the close correlation of these two methods.(118) Since they were measured at the same time we calculated the mean for baseline LVEDD (combined) if both M-Mode and 2D were available. If only one of them existed at baseline this value was used. For the maximal LVEDD (combined) value we only selected the highest value documented during the two-year interval. The LVEDD (combined) maximal value for each patient was also classified according to the categories proposed by the ASE/EACI as described in **table 7** above.

LV Size Category

Left ventricular size was also described using the categories utilized at the LKH Graz. These are:

- Normal left ventricular size
- Borderline left ventricular size
- Mildly enlarged left ventricle
- Mildly-moderately enlarged left ventricle
- Moderately enlarged left ventricle
- Moderately-severely enlarged left ventricle
- Severely enlarged left ventricle

Both the baseline value and the poorest category during the two-year interval were selected and it was determined whether a change of categories took place or not.

2.4.4 Cardiotoxicity

Possible cardiotoxicity was determined based on the difference between the baseline and the minimal LVEF value during the two-year interval after trastuzumab initiation date. Different cut-offs were tested:

- LVEF decrease of 15% absolute
- LVEF decrease of 10% absolute
- LVEF decrease of 15% relative
- LVEF decrease of 10% relative
- LVEF below 54%
- LVEF below 50%
- LVEF decrease of 10% absolute to a value below 54%
- LVEF decrease of 10% absolute to a value below 50%

Patients without baseline LVEF or with only the baseline LVEF available were not included in the calculation of LVEF decrease. They were however included in the analysis whether the LVEF was below 54% or 50% during or after therapy. Patients with only the baseline echocardiography or with no echocardiography at all were excluded from all calculations regarding cardiotoxicity. A LVEF decrease of 10% absolute to a value below 54% was used to define cardiotoxicity in later analyses.

2.4.5 Laboratory Results

The following laboratory parameters were analyzed:

- High sensitive troponin T (hs-TnT)
- NT pro-BNP
- Total cholesterol

Results from blood analysis for cardiac parameters and cardiac risk factors were extracted from the information system MEDOCS. Only results from 90 days before to 455 days after initiation with trastuzumab therapy were considered. Laboratory examinations performed outside the LKH Graz were not considered. If more than one value was available for each patient, the blood analysis closest before or after trastuzumab initiation date was chosen.

2.4.6 Pre-existing Medical Conditions

Pre-existing medical conditions were determined from the diagnoses provided in the medical records of the department of cardiology and oncology. Medical conditions relevant for the cardiovascular system which were present before and during the time of trastuzumab treatment were considered. Additionally, hypercholesterolemia was determined by the evaluation of blood analysis as described above (cut-off value ≥ 200 mg/dl) and overweight/obesity was categorized using the body mass index (cut-off value ≥ 25 kg/m²) according to the WHO classification.(119).

2.4.7 Pharmaceutical Co-Therapy

Information on pharmaceutical co-therapy was retrieved from medical records of the Department of Oncology and the Department of Cardiology and from paper files of the Department of Oncology. Therapy before or during time of trastuzumab treatment was considered. Only cardiovascular medication was evaluated in this thesis.

2.5 Statistical Analysis

For data analysis the program SPSS (IBM SPSS Statistics, Version 23) was used. Data from the final Excel spreadsheet was imported into the program. It was then analyzed using descriptive statistics. For metric variables the minimal and maximal value were stated as well as the mean, standard deviation and median. For nominal variables the absolute and relative frequencies were indicated. Diagrams were created from these results using SPSS and Excel.

A contingency table was created to compare the occurrence of cardiotoxic effects based on LVEF decline and the consequential discontinuation of therapy. For this objective an LVEF decrease of 10 absolute percentage points to a value below 50% was chosen based on the recommendation for termination of therapy as stated in the specialized drug information for Herceptin®.(69)

In a following step the study cohort was divided into two groups according to the occurrence of cardiotoxicity. Here an EF decrease of 10 absolute percentage points to a value below 54% was chosen based on the recommendations for cardiac chamber quantification and the definition of normal LVEF range proposed by Lang et. al.(117) The parameters described above were then determined for both groups and were then analyzed for differences. Metric variables were tested for normal distribution using the Kolmogorov-Smirnov-Test. According to the result the T-Test for independent variables (normal distribution) or Mann-Whitney-U-Test (no normal distribution) was used to compare age, BMI, baseline EF and baseline LVEDD (combined) in both groups. Diagrams were created using SPSS. The distribution of nominal variables such as the individual medical conditions, cardiovascular risk factors and cardiovascular drugs for both groups was presented using contingency tables and diagrams. Chi-square test and (in case of restrictions due to expected counts of less than 5 in more than 20% of cells) Fisher's exact test were used to test whether there was a significant difference between the expected and observed counts. For all statistical analyses $p<0.05$ was determined to indicate significance.

3 Results

3.1 Study Population

The final study population included 202 women. The mean age at therapy begin was 56.1 years (SD=12.1) and the mean BMI 26.0 kg/m² (SD=5.4). A more detailed presentation of age, body length, weight, BMI and Karnofsky performance score (KPS) is presented in **table 8**.

	N	Min.	Max.	Mean	SD	Median
Age (years)	202	27	85	56.11	12.097	55.50
Length (cm)	165	148	185	164.21	6.216	164.00
Weight (kg)	176	47	129	69.37	14.844	65.50
BMI (kg/m²)	164	17.7	42.7	26.016	5.4436	24.705
KPS (%)	175	80	100	96.20	5.896	100.00

Table 8: Age, Length, Weight, BMI and Karnofsky Performance Score of the study population
N = number of patients with valid data, Min. = minimum, Max. = maximum, SD = standard deviation.

3.1.1 Tumor Identity

All patients included in the study were HER2 positive breast cancer patients. The exact cancer location could not be specified from the records that were available to us in 51.0% of cases (n=103). Apart from that the most common localization was the upper outer quadrant with 24.8% (n=50), followed by the lower outer quadrant with 7.4% (n=15) and the upper inner quadrant (6.4%, n=13). 10 patients (5.0%) had a tumor of the central portion of the breast, 6 (3.0%) an overlapping lesion and 5 (2.5%) a tumor of the lower inner quadrant of the breast. Tumor grade was G1 in 2 patients (1.0%), G2 in 68 patients (33.7%) and G3 in 117 patients (57.9%). In 15 cases (7.4%) no information was available. Complete data on the exact tumor stage was only available in 118 patients (58.4%) according to the records that were available to us. It is presented in **table 9**. Details regarding the hormone receptor status and Ki67 proliferation index are presented in **tables 10 and 11**.

Stage	N	Percentage
Stage IA	46	22.8%
Stage IB	5	2.5%
Stage IIA	38	18.8%
Stage IIB	16	7.9%
Stage IIIA	5	2.5%
Stage IIIB	3	1.5%
Stage IIIC	4	2.0%
Stage IV	1	0.5%
Data not available	84	41.6%

Table 9: Tumor stage in the study population

Hormone Receptor Status	ER	PR
Positive*	120 (59.4%)	103 (51.0%)
Negative	71 (35.1%)	85 (42.1%)
Data not available	11 (5.4%)	14 (6.9%)

Table 10: Hormone receptor status in the study population. *without further distinction.
ER = estrogen receptor, PR = progesterone receptor.

Ki67 category	N	Frequency
Low proliferating	30	14.9%
Intermediate proliferating	38	18.8%
Highly proliferating	76	37.6%
Data not available	58	28.7%

Table 11: Proliferation index Ki67 in the study population.

3.1.2 Cancer Therapy

3.1.2.1 Treatment intention

Treatment intention was adjuvant in 55.0% of cases (n=111) and neoadjuvant in 45.0% of cases (n=91).

3.1.2.2 Chemotherapy and Immunotherapy

Trastuzumab mono-therapy was administered in 28 cases (13.9%), the remainder of patients received additional chemotherapy previous or concurrently to trastuzumab. Frequencies for trastuzumab, anthracycline and taxane therapy are given in **figure 4**. Other common chemotherapeutic drugs not considered here were fluorouracil and cyclophosphamide. 24 patients additionally received another antibody (11.9%), whereas 175 (86.6%) did not. In 3 cases (1.5%) no information was available.

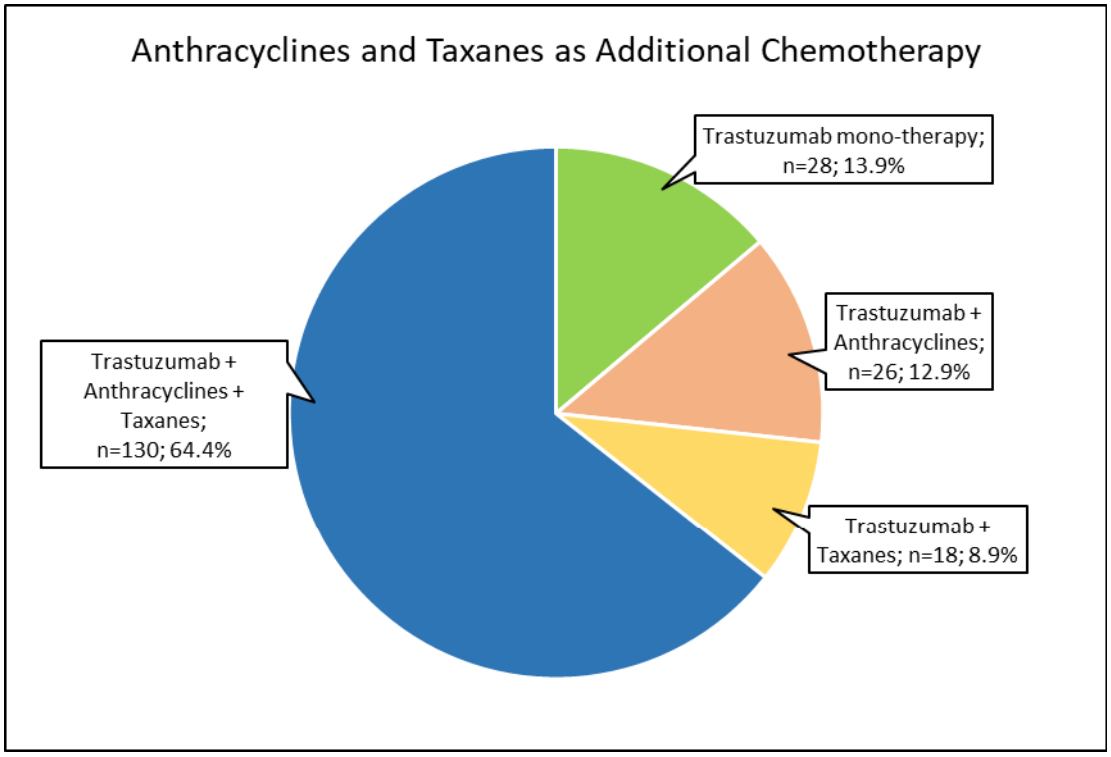

Figure 4: Anthracyclines and taxanes as additional chemotherapy in the study population.

3.1.2.3 Hormonal Therapy and Radiation

Hormonal therapy was administered in 62.9% of cases (n=127) while 75 patients (37.1%) did not receive hormonal therapy. In 74.8% (n=151) cases the therapy regimen included radiation while 51 patients (25.2%) did not receive radiation therapy.

3.1.2.4 Studies

23 Patients were enrolled in a study during cancer treatment (11.4%). 174 patients were not enrolled (86.1%) and in 5 cases (2.5%) data was missing.

3.1.3 Co-morbid Disease

3.1.3.1 Overview

155 patients (76.7%) had at least one cardiovascular risk factor or disease, only in 23.3% (n=47) none was documented (see **table 12**).

Number of Risk Factors	N	Percentage
0	47	23.3%
1	68	33.7%
2	46	22.8%
3	22	10.9%
4	8	4.0%
5	4	2.0%
6	6	3.0%
8	1	0.5%

Table 12: Number of cardiovascular risk factors/diseases in study patients

3.1.3.2 Individual Risk Factors and Diseases

The most frequent cardiovascular co-morbidity documented in medical records was arterial hypertension with 31.2% of patients affected (n=63). A list of all documented pre-existing cardiovascular diseases and risk factors and their frequency in given in **table 13**.

Disease/Risk Factor	N	Percentage
Arterial hypertension	63	31.2%
Smoking	19	9.4%
Obesity	13	6.4%
Diabetes	11	5.4%
St.p. deep vein thrombosis	11	5.4%
Coronary artery disease	7	3.5%
Hypercholesterolemia	6	3.0%
St.p. syncope	6	3.0%
Unspecified heart complaints	6	3.0%
Atrial fibrillation	5	2.5%
St.p. pulmonary embolism	4	2.0%

St.p. Stroke/TIA	4	2.0%
Dilative cardiomyopathy	3	1.5%
Supraventricular tachycardia	3	1.5%
Hyperlipidemia	3	1.5%
Hypertensive crisis	2	1.0%
Pericardial effusion	2	1.0%
Sinus bradycardia	2	1.0%
Atrioventricular block	2	1.0%
Hypertensive heart disease	2	1.0%
St.p. LVEF decrease	2	1.0%
LV Hypertrophy	2	1.0%
Aortic valve stenosis	2	1.0%
St.p. myocarditis	2	1.0%
Atherosclerosis	1	0.5%
Dyspnea	1	0.5%
Mönckeberg medial sclerosis	1	0.5%
Carotid artery stenosis	1	0.5%
Mitral insufficiency	1	0.5%
Tricuspid insufficiency	1	0.5%
Aortic insufficiency	1	0.5%
Left bundle branch block	1	0.5%
Left heart failure	1	0.5%
Atherosclerotic heart disease	1	0.5%
Aortic sclerosis	1	0.5%
Atrial dilatation	1	0.5%
Cardiomegaly	1	0.5%

Table 13: Cardiovascular diseases and risk factors derived from medical records. TIA = transient ischemic attack, St.p. = status post

3.1.3.3 Obesity and Hypercholesterolemia

Apart from documentation in medical records obesity and hypercholesterolemia were also evaluated according to the BMI and lab values of total cholesterol, respectively. Information is presented in **figures 5 and 6**.

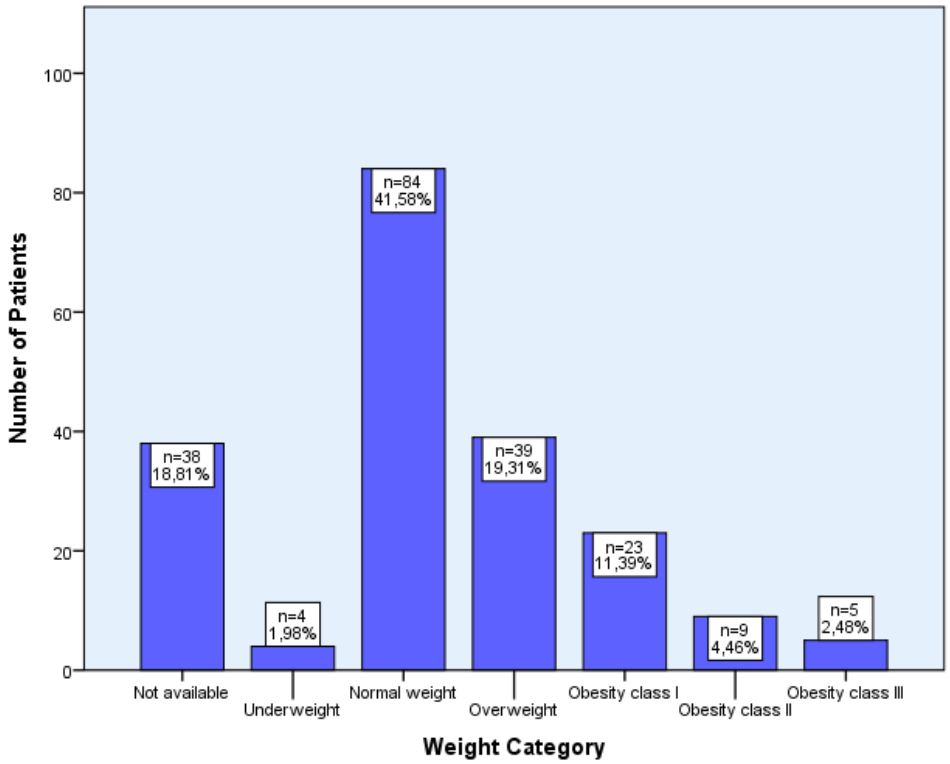

Figure 5: Weight categories derived from BMI.

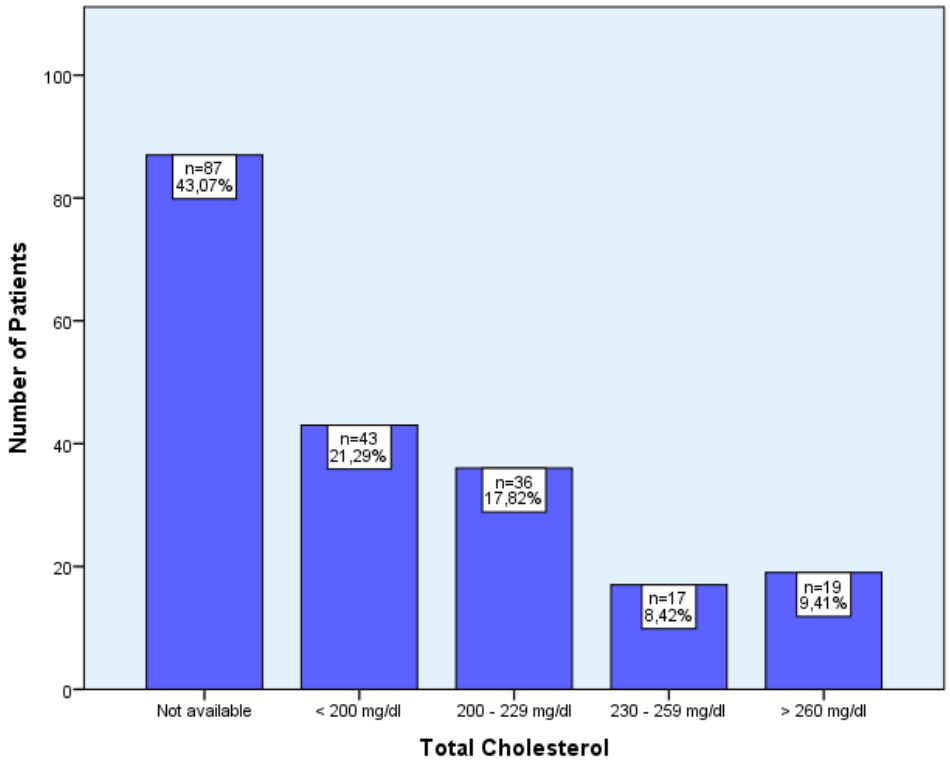

Figure 6: Total cholesterol derived from laboratory values.

3.1.4 Co-Therapy

The most relevant cardiovascular medications included ACE inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), beta blockers (BBs), calcium channel blockers (CCBs), statins, diuretics and any type of diabetes medication. An overview of cardiovascular co-medication is given in **table 14**.

Drug class	Medication	No medication	Not available
ACE inhibitors	35 (17.3%)	156 (77.2%)	11 (5.4%)
ARBs	15 (7.4%)	176 (87.1%)	11 (5.4%)
Beta blockers	47 (23.3%)	144 (71.3%)	11 (5.4%)
Statins	25 (12.4%)	166 (82.2%)	11 (5.4%)
Nitroglycerin	4 (2.0%)	187 (92.6%)	11 (5.4%)
Diabetes medication	9 (4.5%)	182 (90.1%)	11 (5.4%)
Hydrochlorothiazide	41 (20.3%)	150 (74.3%)	11 (5.4%)
Loop diuretics	11 (5.4%)	180 (89.1%)	11 (5.4%)
Spironolactone	5 (2.5%)	186 (92.1%)	11 (5.4%)
Calcium channel blockers	19 (9.4%)	172 (85.1%)	11 (5.4%)

Table 14: Cardiovascular co-medication in study patients. ARBs = angiotensin II receptor blockers

3.1.5 Cardiac Biomarkers

High sensitive Troponin T was only determined once in 10 patients (5.0%) and twice in two patients (1.0%). In 190 patients (94.1%) it was never determined. NT pro-BNP was determined once in 26 patients (12.9%) and more than once in 10 patients (5.0%). It was never determined in 166 patients (82.2%).

3.2 Trastuzumab and Cardiotoxicity

3.2.1 Discontinuation of Trastuzumab Therapy

166 out of 202 patients (82.2%) received full treatment with trastuzumab, whereas treatment was stopped ahead of time in 36 patients (17.8%). In 24 cases (11.9%) early termination of trastuzumab therapy was due to cardiovascular adverse events, in 12 cases (5.9%) due to other reasons. If trastuzumab was discontinued due to cardiac reasons it was after a mean of 7.41 months (SD=3.46). A detailed list of reasons is given in **table 15.**

Reason for discontinuation of therapy	**N**	**Percentage**
Cardiac:		
EF decrease	17	8.4%
EF decrease and suspected CAD	1	0.5%
Cardiotoxicity, not further specified	3	1.5%
Breathing difficulties and palpitations	1	0.5%
Incipient toxic cardiomyopathy	1	0.5%
Tachycardic dysrhythmia	1	0.5%
Non-cardiac:		
Patient's wish/refusal	5	2.5%
Therapy adjustment	2	1.0%
Anaphylactic reaction	1	0.5%
Joint disease	1	0.5%
Ear/nose/throat side effects	1	0.5%
Incarcerated hernia	1	0.5%
Intolerance and general side effects	1	0.5%

Table 15: Reasons for discontinuation of trastuzumab therapy. CAD = coronary artery disease

Time of Discontinuation

In those cases where trastuzumab was discontinued due to cardiac reasons therapy was stopped during the first quarter of treatment in 4 patients, during the second quarter in 5 patients, during the third quarter in 5 patients and during the last quarter of therapy in 10 patients. A more detailed overview is given in **figure 7**.

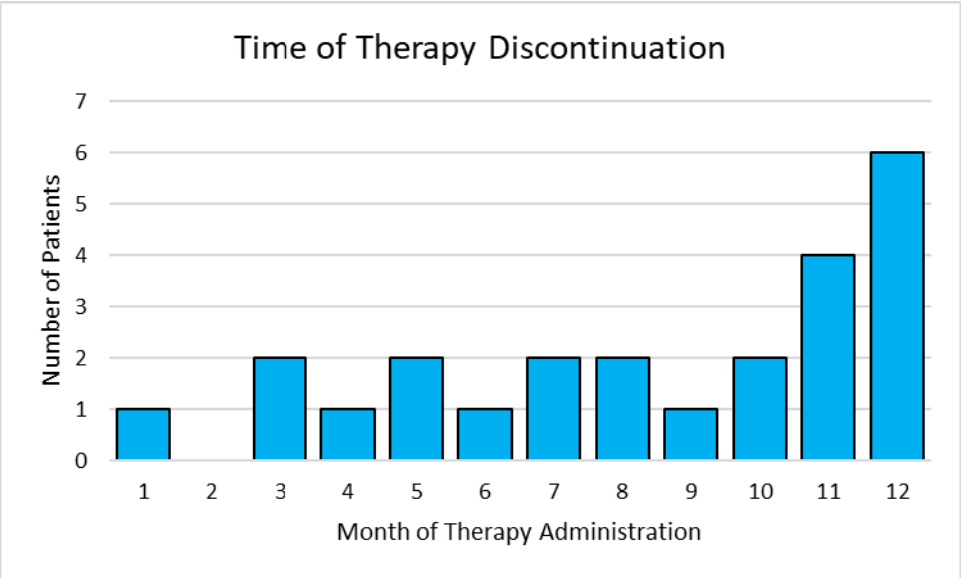

Figure 7: Time of therapy interruption in case of cardiotoxicity.

Reversibility of Cardiotoxicity

In two patients trastuzumab was discontinued due to dysrhythmia and palpations while the remaining patients suffered from LVEF decreases. Out of these 22 patients 13 (59.1%) had a LVEF of at least 54% at some point during follow-up while 9 patients (40.9%) remained below this value. Considering the differing ASE/EACVI classification cardiotoxic effects were reversible in 15 patients (68.2%), partially reversible in two patients (9.1%) and irreversible in 5 patients (22.7%). The period of observation after discontinuation of therapy varied among the patients.

3.2.2 Echocardiography

The number of echocardiographic results that was available for each patient in the two-year interval (including baseline) differed from none to 14 as can be seen in **table 16**. 82.6% had 4 or more echocardiographies.

Number of Echocardiographies	N	Percentage
0	5	2.5%
1	10	5.0%
2	8	4.0%
3	12	5.9%
4	41	20.3%
5	51	25.2%
6	40	19.8%
7 - 14	35	17.3%

Table 16: Number of echocardiographies available for each patient.

3.2.3 Effects on Left Ventricular Function

3.2.3.1 LVEF

Mean EF at baseline was 63.1% (SD= 6.8). For the lowest EF that was measured for each patient during the two years after initiation of trastuzumab therapy the mean was 57.6% (SD= 6.9). More detailed parameters are shown in **table 17**.

	N	Min.	Max.	Mean	SD	Median
EF baseline	181	50	82	63.041	6.8325	62.000
EF minimum	195	35	76	57.459	6.7298	59.000

Table 17: Baseline and minimal LVEF after trastuzumab initiation. Min. = minimum, Max. = maximum

On average a LVEF decline of 5.84% was observed (SD=7.20). The mean LVEF decrease in the group that completed therapy was 4.66% (SD=6.44), it was 4.22% (SD=6.51) in those where trastuzumab was discontinued because of non-cardiac reasons and 13.60% (SD=7.23) in the group in which trastuzumab was discontinued because of cardiotoxicity.
According to the classification of the ASE/EACVI the minimal LVEF value was in normal range for 153 patients (75.7%), mildly abnormal in 36 cases (17.8%) and moderately abnormal in 6 cases (3.0%). No data was available in 7 cases (3.5%). More detailed information on LVEF decrease is presented in **table 18**.

	Affected	Not affected	Not available
LVEF < 54%	42 (20.8%)	153 (75.7%)	7 (3.5%)
LVEF < 50%	20 (9.9%)	175 (86.6%)	7 (3.5%)
LVEF decrease 15% absolute	21 (10.4%)	158 (78.2%)	23 (11.4%)
LVEF decrease 10% absolute	50 (24.8%)	129 (63.9%)	23 (11.4%)
LVEF decrease 15% relative	44 (21.8%)	135 (66.8%)	23 (11.4%)
LVEF decrease 10% relative	74 (36.6%)	105 (52.0%)	23 (11.4%)
LVEF -10% abs. to a value <54%	25 (12.4%)	154 (76.2%)	23 (11.4%)
LVEF -10% abs. to a value <50%	18 (8.9%)	161 (79.7%)	23 (11.4%)

Table 18: LVEF decrease among study patients.

3.2.3.2 LVF Category

At baseline 175 patients (87.5%) had normal left ventricular function according to the categories used at the LKH Graz. Considering the poorest left ventricular function documented during the two-year interval 151 patients (75.5%) had normal systolic function. Exact data is shown in **table 19**.

LV Systolic Function	Baseline	Minimum
Hyperdynamic	-	1 (0.5%)
Normal	177 (87.6%)	150 (74.3%)
Borderline	4 (2.0%)	12 (5.9%)
Mildly-impaired	-	25 (12.4%)
Mildly – to moderately impaired	-	1 (0.5%)
Moderately impaired	-	6 (3.0%)
Moderately – severely impaired	-	2 (1.0%)
Data not available	21 (10.4%)	5 (2.5%)

Table 19: Baseline and poorest LVF category during trastuzumab therapy

In 47 patients (23.3%) a change of the baseline category could be observed during the two-year interval. No data was available in 23 cases (11.4%) and in 132 cases (65.3%) the category did not change over time. Considering only the cases with a dynamic there was a mean change of 2.02 categories (median=2.00) based on the categories described above.

3.2.4 Effects on Left Ventricular Size

3.2.4.1 LVEDD

The mean value for the combined LVEDD at baseline was 44.2 mm (SD=5.2). The combined maximum value during the two-year interval was 50.5 mm (SD=5.6). More information on M-Mode LVEDD, LV2D and the combined value is given in **table 20**.

	N	Min.	Max.	Mean	SD	Median
LVEDD baseline	68	33	74	46.54	5.941	45.00
LV2D baseline	79	32	56	42.68	5.401	42.00
Baseline combined	98	34	60	44.37	5.376	43.75
LVEDD maximum	156	32	74	50.44	6.056	50.00
LV2D maximum	149	32	61	46.90	5.326	47.00
Maximum combined	166	35	74	50.59	5.890	50.00

Table 20: Baseline and maximum LV during trastuzumab therapy. All dimensions in millimeters.

Considering the maximum combined value for left ventricular dimension 107 patients were in the normal range (53.0%) according to the ASE/EACVI. 33 patients (16.3%) had mildly abnormal, 22 patients (10.9%) moderately abnormal and 4 patients (2.0%) severely abnormal LVEDD dimensions. In 36 cases (17.8%) no data was available.

3.2.4.2 LV Size Category

LV size category according to those used at the LKH Graz changed in 17 patients (8.4%) with a median of 1.71 categories (median=2.00). In 152 patients (75.2%) it remained the same while in 33 cases (16.3%) no data was available. Data is presented in **table 21**.

LV Size	Baseline		Maximum	
Small-luminal left ventricle	-		1	(0.5%)
Normal	165	(81.7%)	173	(85.6%)
Borderline	-		8	(4.0%)
Mildly enlarged	3	(1.5%)	11	(5.4%)
Mildly – to moderately enlarged	-		2	(1.0%)
Data not available	34	(16.8%)	7	(3.5%)

Table 21: Baseline LV size and poorest category during trastuzumab therapy.

3.2.5 Cardiotoxicity and Discontinuation of Therapy

18 patients experienced an absolute LVEF decrease of 10% to a value below 50%. In these patients, treatment with trastuzumab was discontinued because of cardiotoxicity in 15 cases (83.3%). In one case (5.6%) treatment was adapted due to progression of disease. Two patients (11.1%) received full therapy, one of these experienced the LVEF decrease at completion of therapy. In the group of 161 patients without such LVEF decrease therapy was discontinued for cardiac reasons in nine cases (5.6%) and for non-cardiac reasons in eight cases (5.0%). 144 of these patients (89.4%) received full therapy (see **figure 8**).

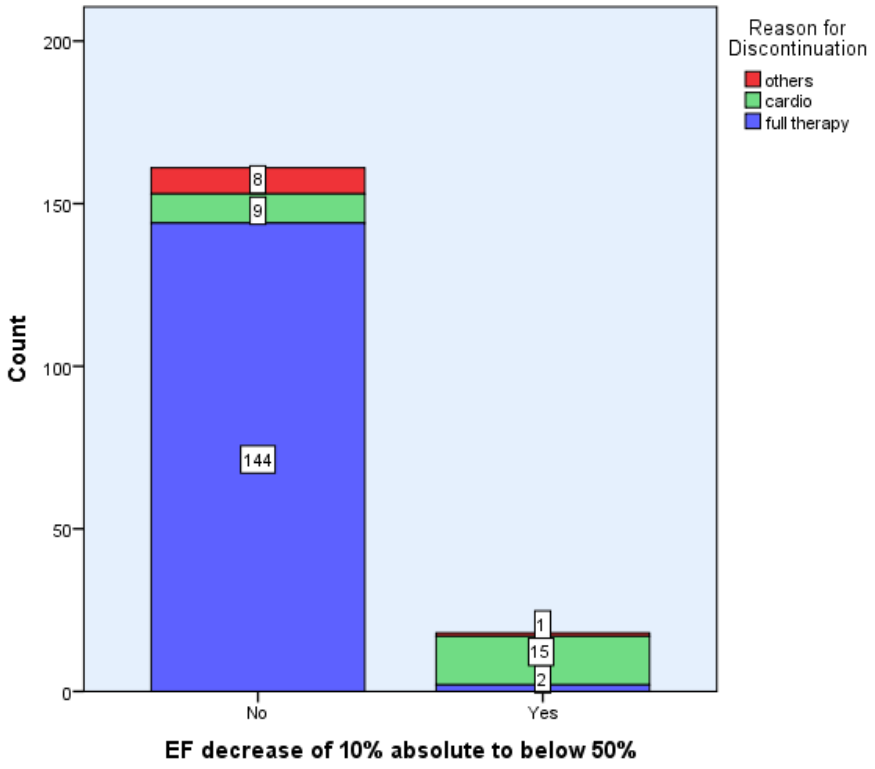

Figure 8: LVEF decrease and completion of therapy.

3.3 Differences between both groups

3.3.1 Age

In 179 patients data was available for both age and LVEF change. Patients who developed cardiotoxicity were older (M=59.00 years, SD 10.66, n=25) than patients who did not experience cardiotoxicity (M=55.05 years, SD=11.73, n=154). Mean difference between both groups was 3.96 years (more detailed information in **figure 9**).

T-test for independent variables showed that the difference between age in both groups was not significant (t(177)= -1.582, p=0.115).

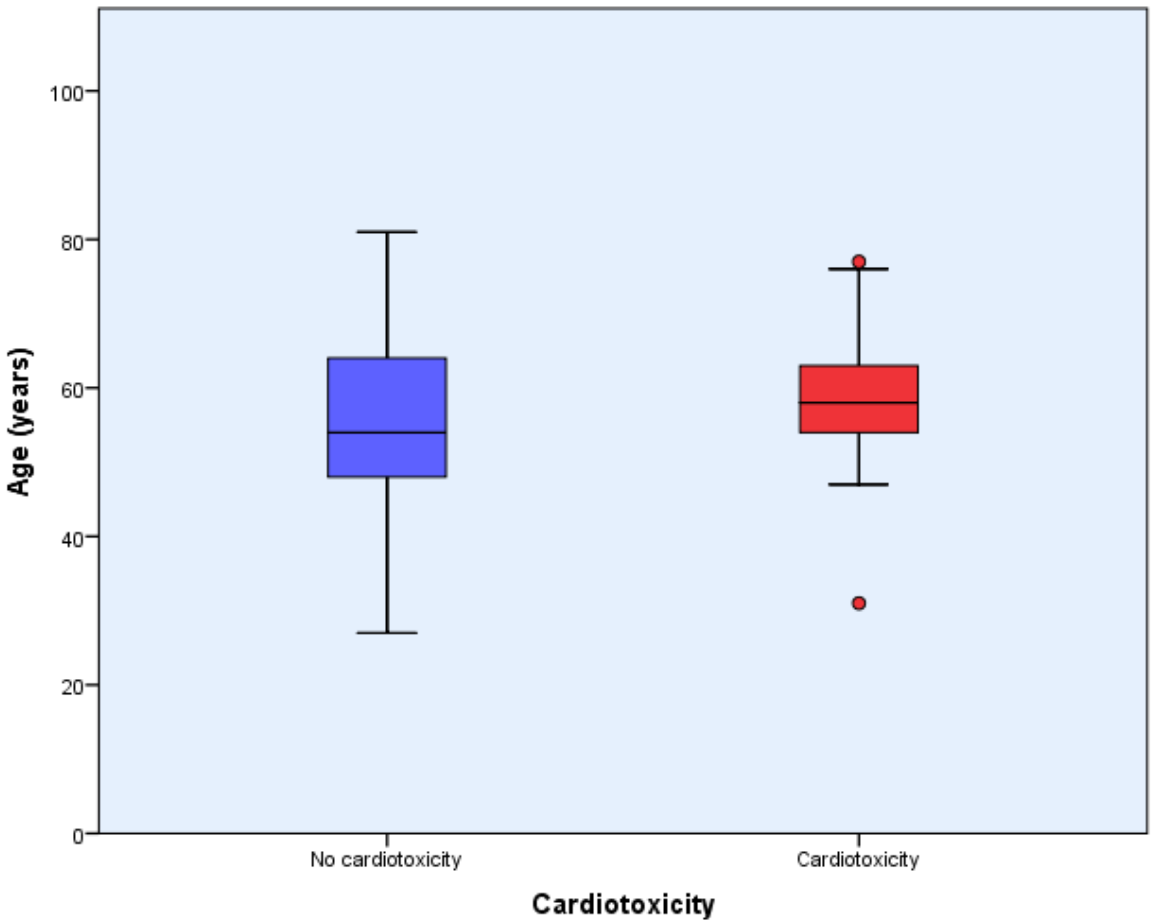

Figure 9: Age of patients with and without cardiotoxicity.

3.3.2 Body Mass Index

For 150 patients data was available for both BMI and LVEF change. Patients who developed cardiotoxicity were had a higher BMI (M=26.39 kg/m², SD 4.48, n=23) than patients who did not experience cardiotoxicity (M=25.85 kg/m², SD=5.72, n=127).

The median BMI was 24.91 in patients who experienced cardiotoxicity and 24.17 in patients without cardiotoxicity. A graphic account can be seen in **figure 10**.

Mean rank was 74.11 in patients without cardiotoxicity and 83.15 in patients with cardiotoxicity. Mann-Whitney-U-test showed that the difference between BMI in both groups was not significant (U=1284.500, Z= -0.918, p=0.359).

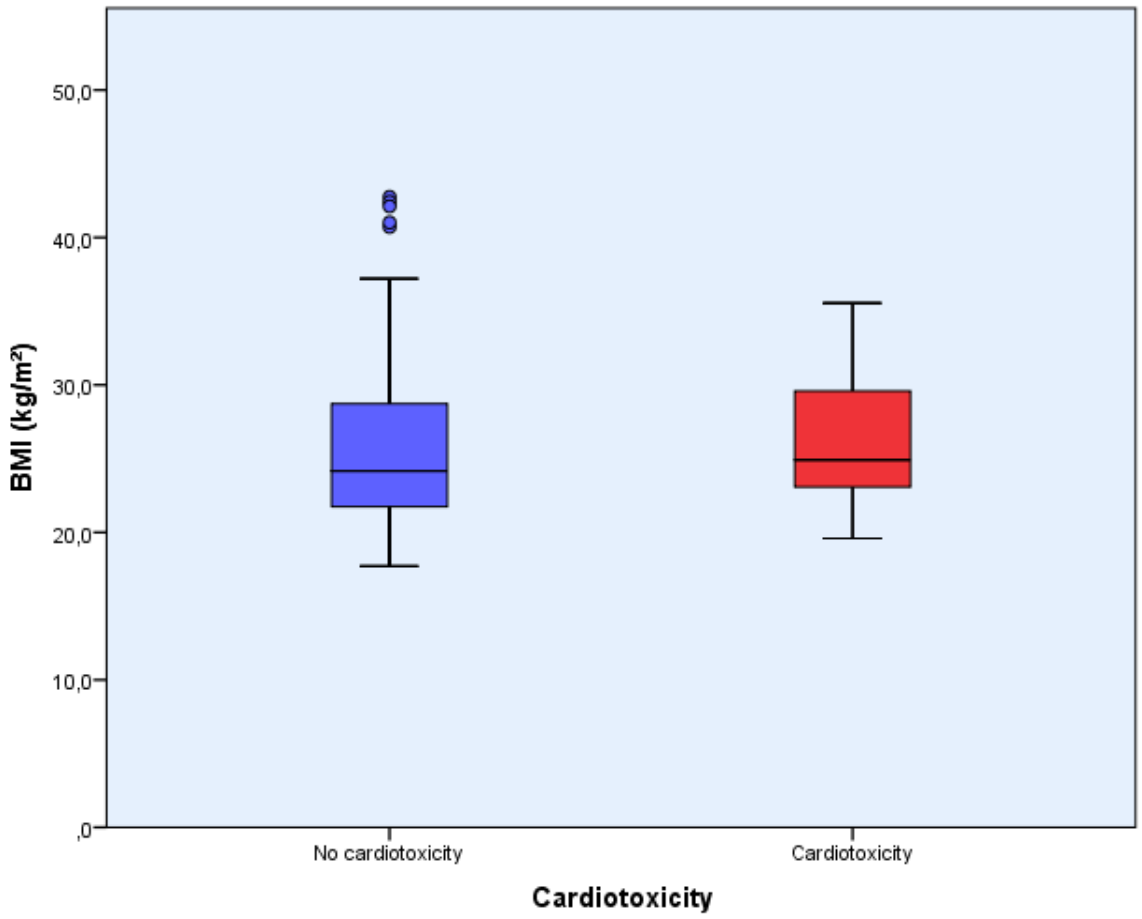

Figure 10: BMI of patients with and without cardiotoxicity.

3.3.3 Baseline LVEF

Sufficient data was available for 179 patients. Baseline LVEF was lower in patients who developed cardiotoxicity (M=61.36, SD=5.96, n=25) than in patients without cardiotoxicity (M=63.24, SD=6.84, n=154). The median for baseline LVEF was 62.00 in patients who experienced cardiotoxicity and 63.50 in patients without cardiotoxicity. A graphic account can be seen in **figure 11**.

Mean rank was 92.08 in patients without cardiotoxicity and 77.16 in patients with cardiotoxicity. Mann-Whitney-U-test showed that the difference between baseline LVEF in both groups was not significant (U=1604.000, Z= -1.340, p=0.180).

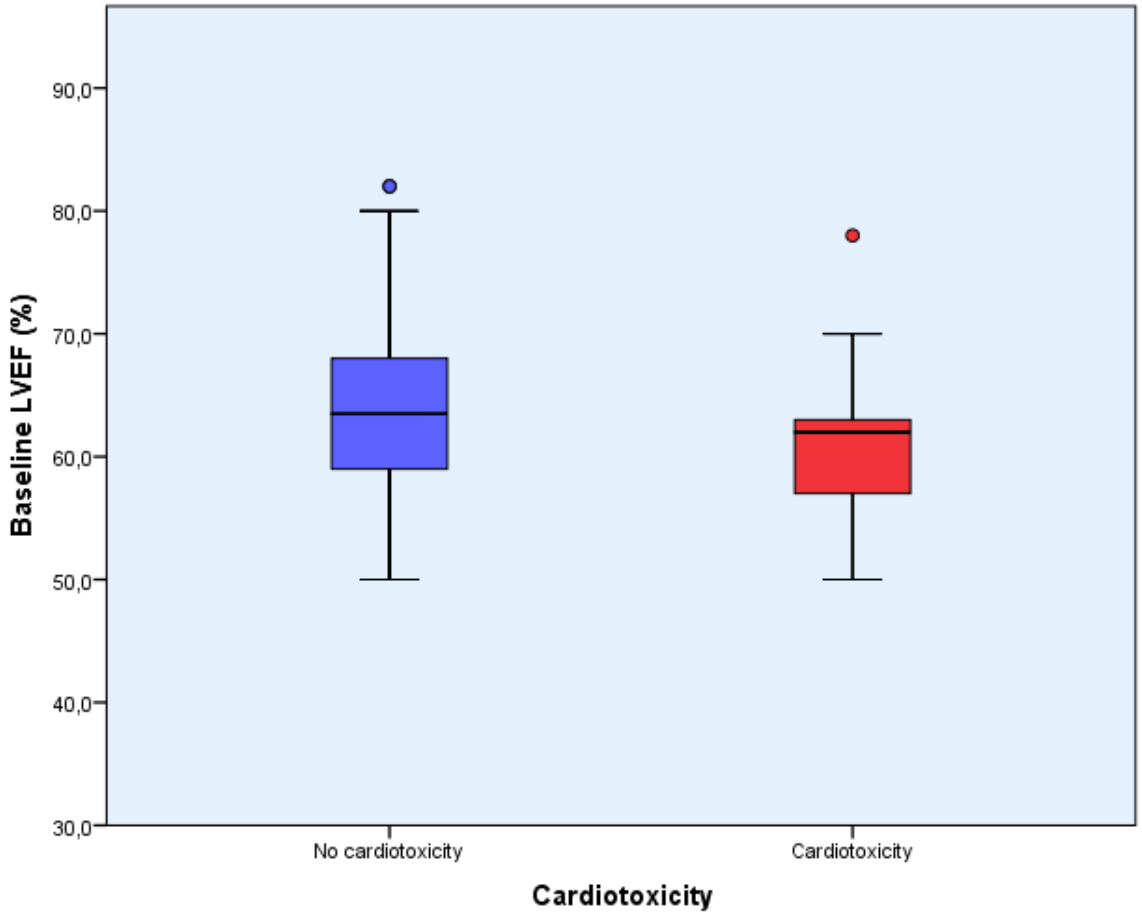

Figure 11: Baseline LVEF of patients with and without cardiotoxicity

3.3.4 Baseline LVEDD

For 96 patients, data was available for both baseline LVEDD and LVEF change. Patients with cardiotoxicity had larger LV end-diastolic dimensions (M=48 mm, SD=5.36, n=12) at baseline than patients without cardiotoxicity (M=43.86, SD=5.17, n=84). Mean difference between both groups was 4.18 mm. The distribution of LVEDD at baseline is shown in **figure 12**. Data refers to the LVEDD combined value.

T-test analysis showed that the difference between baseline combined LVEDD in both groups is significant (t(94)= -2.608, p=0.011). Effect size according to Cohen's d is 0,793 which implies a moderate effect.

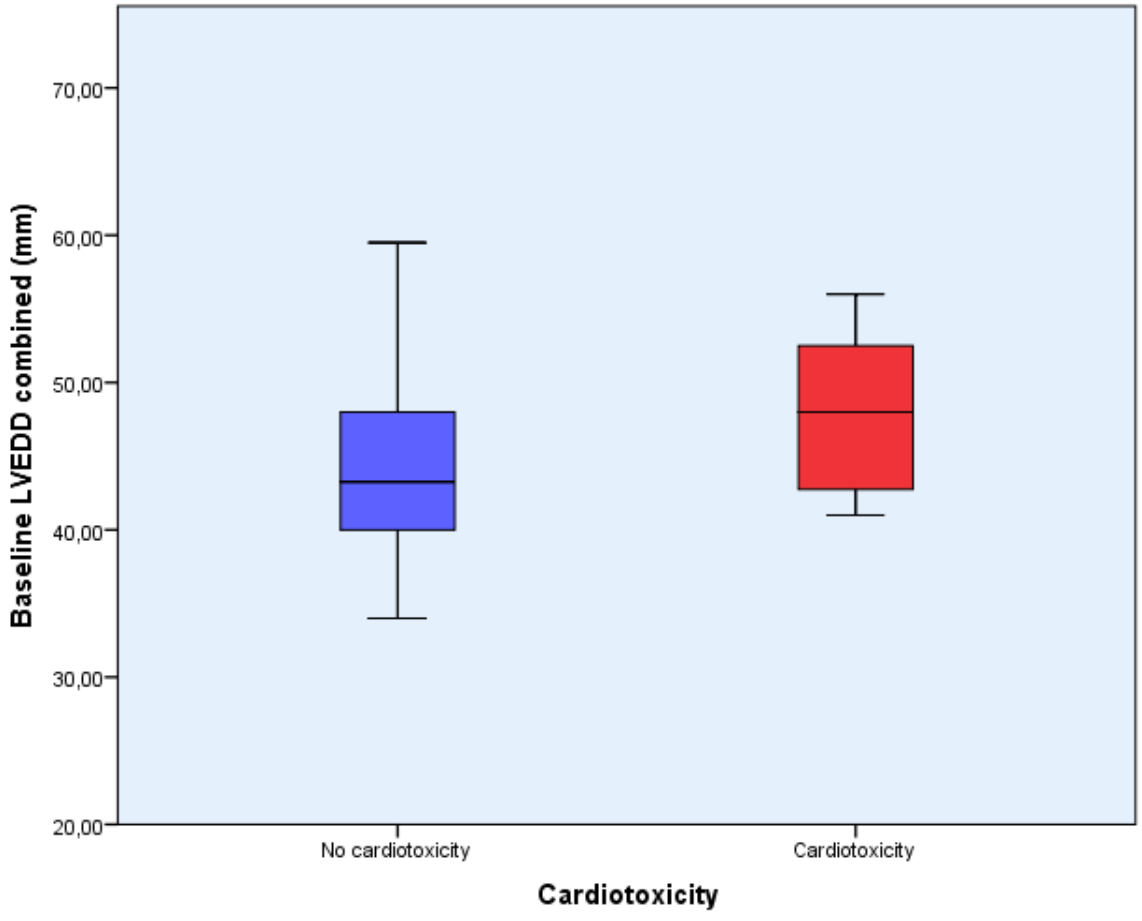

Figure 12: Baseline LVEDD combined of patients with and without cardiotoxicity.

3.3.5 Cardiovascular Diseases and Risk Factors

For 179 patients, data was available for both co-morbid disease and LVEF change. Data for lab values of total cholesterol were only available in 97 cases. An overview of the percentage of women affected by each disease in both groups is presented in **figure 13**. No statistically significant difference between the expected and observed counts regarding the single pre-existing diseases and cardiotoxicity could be found.

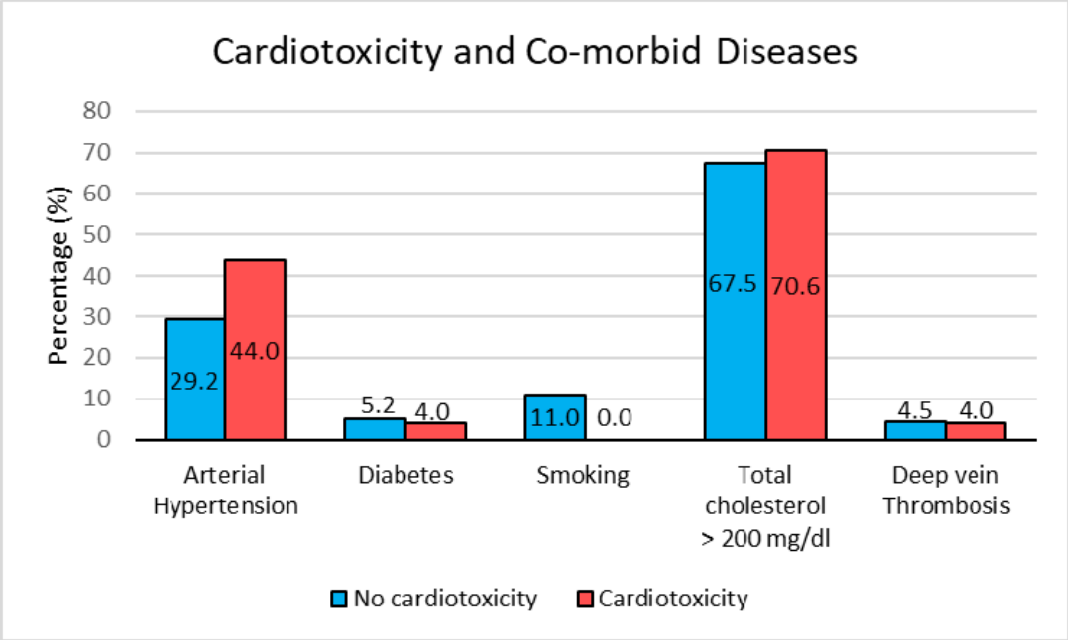

Figure 13: Co-morbid diseases in patients with and without cardiotoxicity.

3.3.5.1 Arterial Hypertension

The exact number of patients affected by arterial hypertension in each group are presented in **table 22** below. Chi-square analysis did not show a significant difference between expected and observed counts in patients with or without arterial hypertension and cardiotoxicity ($\chi^2(1, n=179)=2.185$, $p=0.139$).

	No arterial HT	**Arterial HT**	**Total**
No cardiotoxicity	109	45	154
Cardiotoxicity	14	11	25
Total	123	56	179

Table 22: Counts for arterial hypertension in patients with and without cardiotoxicity.

3.3.5.2 Diabetes

Table 23 shows the exact counts for patients affected by diabetes in both groups. According to Fisher's exact test the difference between expected and observed counts in both groups is not statistically relevant (p=1.000).

	No diabetes	**Diabetes**	**Total**
No cardiotoxicity	146	8	154
Cardiotoxicity	24	1	25
Total	170	9	179

Table 23: Counts for diabetes in patients with and without cardiotoxicity.

3.3.5.3 Smoking

Table 24 shows the number of patients who were smokers in each group. Fisher's exact test shows no significant difference between distribution among both variables (p=0.135).

	Non-smoker	**Smoker**	**Total**
No cardiotoxicity	137	17	154
Cardiotoxicity	25	0	25
Total	162	17	179

Table 24: Counts for smoking in patients with and without cardiotoxicity.

3.3.5.4 Hypercholesterolemia

The exact number of patients affected by hypercholesterolemia > 200 mg/dl in each group is presented in **table 25**. Chi-squared test shows no significant difference between expected and observed counts ($\chi^2(1, n=97)=0.61$, p=0.804).

	Hypercholesterolemia	**Hypercholesterolemia**	**Total**
No cardiotoxicity	26	54	80
Cardiotoxicity	5	12	17
Total	31	66	97

Table 25: Counts for hypercholesterolemia in patients with and without cardiotoxicity.

3.3.5.5 Deep vein thrombosis

Table 26 shows the number of patients who had a deep vein thrombosis (DVT) in each group. Fisher's exact test shows no significant difference between distribution among both variables (p=1.000).

	No DVT	**DVT**	**Total**
No cardiotoxicity	147	7	154
Cardiotoxicity	24	1	25
Total	171	8	179

Table 26: Counts for deep vein thrombosis in patients with and without cardiotoxicity.

3.3.6 Cardiovascular Medication

For 171 patients, data was available for both medication and LVEF change. Differences regarding cardiovascular co-therapy in both groups are presented in **figures 14 and 15**. The percentage of women with ACEIs and calcium channel blockers is higher in the cardiotoxicity group, whereas statins, loop diuretics and spironolactone are slightly higher in the group without cardiotoxicity. The rates for ARBs, BBs, HCT, diabetes medication and nitrates are similar between patients with and without cardiotoxicity. A statistically significant difference between expected and observed counts in both groups could only be found for ACE-inhibitors. Details on exact counts are given in the following sub-chapters.

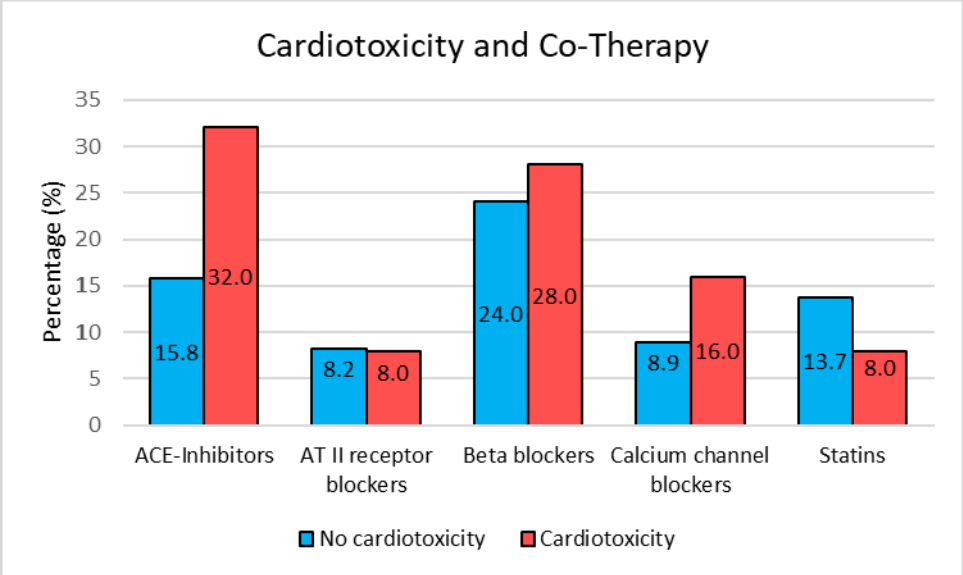

Figure 14: Co-medication in patients with and without cardiotoxicity (1)

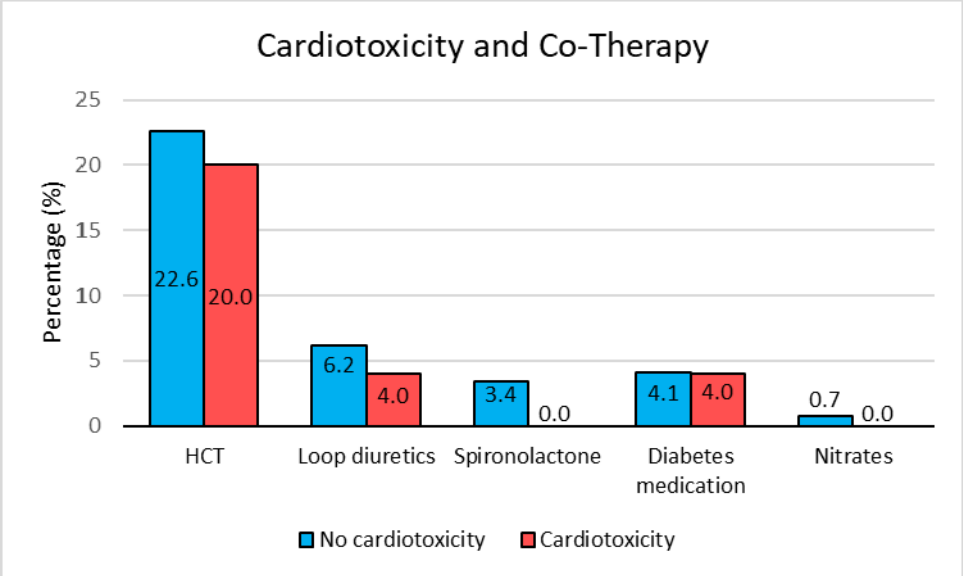

Figure 15: Co-medication in patients with and without cardiotoxicity (2)

3.3.6.1 ACE-Inhibitors

The number of women with and without ACE-Inhibitor medication in both groups is shown in **table 27**. Fisher's exact test showed that the difference between expected and observed counts in this case was significant ($p<0.049$).

	No ACE-Inhibitors	**ACE-Inhibitors**	**Total**
No cardiotoxicity	124	22	146
Cardiotoxicity	17	8	25
Total	141	30	171

Table 27: Counts for ACE-Inhibitors in patients with and without cardiotoxicity.

3.3.6.2 AT II receptor blockers

The number of patients with and without ARBs in both groups is presented in **table 28**. According to Fisher's exact test there was no significant difference between expected and observed counts ($p=1.000$).

	No ARBs	**ARBs**	**Total**
No cardiotoxicity	134	12	146
Cardiotoxicity	23	2	25
Total	157	14	171

Table 28: Counts for ARBs in patients with and without cardiotoxicity.

3.3.6.3 Beta Blockers

Table 29 shows the exact number of patients with and without beta blockers in both groups. Chi-squared test did not show a significant difference between expected and observed counts (($\chi^2(1, n=171)=0{,}187$, $p=0.666$).

	No beta blockers	**Beta blockers**	**Total**
No cardiotoxicity	111	35	146
Cardiotoxicity	18	7	25
Total	129	42	171

Table 29: Counts for beta blockers in patients with and without cardiotoxicity.

3.3.6.4 Calcium channel blockers

The number of patients with and without calcium channel blockers is presented in **table 30**. Fisher's exact test did not show a significant difference between expected and observed counts for these variables (p=0.280).

	No CCBs	**CCBs**	**Total**
No cardiotoxicity	133	13	146
Cardiotoxicity	21	4	25
Total	154	17	171

Table 30: Counts for CCBs in patients with and without cardiotoxicity. *CCB = calcium channel blocker

3.3.6.5 Statins

The number of patients with and without statins in both groups is presented in **table 31**. Fisher's exact test did not show a significant difference between expected and observed counts for these variables (p=0.746).

	No statins	**Statins**	**Total**
No cardiotoxicity	126	20	146
Cardiotoxicity	23	2	25
Total	149	22	171

Table 31: Counts for statins in patients with and without cardiotoxicity.

3.3.6.6 Hydrochlorothiazide

The number of patients with and without hydrochlorothiazide in both groups is presented in **table 32**. Chi-squared test did not show a significant difference between expected and observed counts (($\chi^2(1, n=171)=0{,}084$, p=0.772).

	No HCT	**HCT**	**Total**
No cardiotoxicity	113	33	146
Cardiotoxicity	20	5	25
Total	133	38	171

Table 32: Counts for HCT in patients with and without cardiotoxicity.

3.3.6.7 Loop Diuretics

Table 33 shows the exact number of patients with and without loop diuretics in both groups. Fisher's exact test did not show a significant difference between expected and observed counts (p=1,000).

	No loop diuretics	Loop diuretics	Total
No cardiotoxicity	137	9	146
Cardiotoxicity	24	1	25
Total	161	10	171

Table 33: Counts for loop diuretics in patients with and without cardiotoxicity.

3.3.6.8 Spironolactone

The number of patients with and without spironolactone is shown in **table 34**. Fisher's exact test did not show a significant difference between expected and observed counts (p=1,000).

	No spironolactone	Spironolactone	Total
No cardiotoxicity	141	5	146
Cardiotoxicity	25	0	25
Total	166	5	171

Table 34: Counts for spironolactone in patients with and without cardiotoxicity.

3.3.6.9 Diabetes Medication

The number of patients with and without diabetes medication is presented in **table 35**. Fisher's exact test showed no significant difference between expected and observed counts (p=1.000).

	No diabetes medication	Diabetes medication	Total
No cardiotoxicity	140	6	146
Cardiotoxicity	24	1	25
Total	164	7	171

Table 35: Counts for diabetes medication in patients with and without cardiotoxicity.

3.3.6.10 Nitrates

Only one patient with available data on LVEF change had documented nitrate medication, as can be seen in **table 36**. Fisher's exact test did not show a significant difference between expected and observed counts (p=1.000).

	No nitrates	**Nitrates**	**Total**
No cardiotoxicity	145	1	146
Cardiotoxicity	25	0	25
Total	170	1	171

Table 36: Counts for nitrates in patients with and without cardiotoxicity.

3.3.7 Additional Chemotherapy

The additional chemotherapy regarding anthracyclines and taxanes which patients received was similar between those who later developed cardiotoxicity and those who did not. In the cardiotoxicity group slightly more patients received trastuzumab mono-therapy. The percentage of women who received anthracyclines prior to trastuzumab therapy is equal in both groups. Data is presented in **table 37**.

Therapy regimen (n=179)	**No cardiotoxicity** (n=154)	**Cardiotoxicity** (n=25)
Trastuzumab mono-therapy (n=21, 11.7%)	17 (81.0%)	4 (19.0%)
Trastuzumab + Anthracyclines (n=23, 12.9%)	20 (87.0%)	3 (13.0%)
Trastuzumab + Taxanes (n=14, 7.8%)	13 (92.9%)	1 (7.1%)
Trastuzumab + Anthracyclines + Taxanes (n=121, 67.6%)	104 (86.0%)	17 (14.0%)

Table 37: Additional chemotherapy in patients with and without cardiotoxicity. Percentages in the first column refer to the total cohort, percentages in the second and third column refer to each therapeutical sub-group.

Analyzing the groups according to therapy regimen the incidence of cardiotoxicity was 19.0% in women who received trastuzumab mono-therapy (4 of 21), 13.0% in women who received therapy with trastuzumab and anthracyclines (3 of 23), 7.1% in women with trastuzumab and taxanes (1 of 14) and 14.0% in women who received therapy with

trastuzumab, anthracyclines and taxanes (17 of 121). Cardiotoxicity rates were similar in patients who were treated in a neoadjuvant or adjuvant manner (13.3% or 11 of 83 vs. 14.6% or 14 of 96).

4 Discussion

The aim of this retrospective study was to get an overview of cardiac function during trastuzumab therapy in patients with breast cancer at the LKH Graz and to assess co-morbid disease and co-therapy. We used echocardiographic indices to identify cardiotoxicity. According to the occurrence of cardiotoxicity two groups were determined and in a following step differences between both of them were analyzed. Patients who developed cardiotoxicity had significantly higher baseline LVEDD values and were on ACE-inhibitor medication significantly more often. Although minor differences could be observed for age, BMI, baseline LVEF and certain types of co-therapy and co-morbid disease, none of them reached statistical significance.

4.1 Cardiotoxicity

Discontinuation of Therapy

166 patients received full therapy, whereas trastuzumab was discontinued for cardiac reasons in 24 cases (11.9%) and for non-cardiac reasons in 12 cases (5.9%). Non-cardiac reasons included anaphylactic reaction, joint disease, ear/nose/throat side effects, not further specified general side effects, necessity of surgery for an incarcerated hernia without resumption of therapy, patient's wish and change of therapy regimen. The main cardiac reason which led to termination of trastuzumab therapy was LVEF decrease, however, tachycardic arrhythmias and palpations with dyspnea were also stated.

The rate of therapy interruption due cardiac reasons we observed (11.9%) was higher than the rate of 4.3% described in the multicenter HERA clinical trial.(37) Criteria for therapy discontinuation in this trial were congestive heart failure (CHF) or a confirmed significant LVEF decrease. A significant LVEF decrease was defined as a decline of at least 10 absolute percentage points from baseline and to a value below 50%. In this case treatment was paused until reevaluation three weeks later. If the LVEF was below 45% or between 45 and 49% and still 10 or more absolute percentage points from baseline therapy was discontinued permanently.(37). This approach basically follows the recommendations given in the official package insert.(69) Patients in the HERA trial were younger (49 ± 10 years) than our study population (56 ± 12 years) and seemed to have active cardiovascular disease less frequent. The percentage of women with arterial hypertension was lower than in our study group (17% vs. 31%). Patients with relevant cardiovascular disease including

history of CHF, coronary artery disease, uncontrolled, high-risk arrhythmias or clinically significant valvular disease and those with a baseline LVEF below 55% were excluded from participation in the HERA study. This could be an explanation for the difference in discontinuation rates. Also, it is unclear whether strict adherence to official recommendations regarding discontinuation of therapy was present in our study cohort or if other factors that could not be identified retrospectively influenced decision-making in the clinical setting. In a retrospective study similar to this one Yu et al. found a discontinuation rate of 18% in women with adjuvant trastuzumab treatment which implies that there are great differences between clinical trial situations and clinical practice.(120)

Cardiac adverse events that led to discontinuation of trastuzumab treatment could be observed at any time during therapy ranging from shortly after the initial dose up to the last month of therapy. Trastuzumab was discontinued due to cardiac reasons during the first quarter of treatment in four patients, during the second quarter in five patients, during the third quarter in five patients and during the last quarter of treatment in 10 patients. This indicates that even good tolerability at the beginning of therapy does not protect from cardiotoxic effects during the course of treatment.

13 out of 22 patients who suffered an LVEF drop showed a LVEF of at least 54% at some point of time after therapy discontinuation. Still the recovery rate observed in our study is lower than the one observed in the HERA trial (59.1% vs. 69%) although recovery is similarly defined as LVEF of at least 55% at some point after the cardiac end point in that trial.(37) With the data available it cannot be determined whether this difference is real or just an effect due to a short follow-up period or missing control echocardiographies in our study population.

Retrospective Echocardiographic Evaluation

In the retrospective analysis of echocardiographic results 18 patients (8.9%) were affected by an LVEF drop of 10 absolute percentage points to a value below 50%, which is the cut-off to consider therapy discontinuation given in the package insert. It needs to be kept in mind that the definition of cardiotoxicity used to determine the two sub-groups in this study differs from that recommendation. Based on significant LVEF decrease which we defined as decrease of 10 or more absolute percentage points to a value below 54%, 12.4% of our study population suffered from trastuzumab-induced cardiac dysfunction. However, considering each of those variables separately we found that 42 patients (20.8%) had a

LVEF below 54% at some point and that a LVEF decrease of 10% or more could be found in 50 patients (24.8%). This means that up to a quarter of patients may be affected by milder forms of cardiotoxicity. Our results are similar to those that have been published by other authors before. Tarantini et al. found cardiotoxicity defined as LVEF decrease of at least 10% or CHF in up to 27% of patients with adjuvant trastuzumab therapy.(121) Similarly Piotrowski et al. found a cardiotoxicity rate of 20.55% based on total LVEF decrease of 15% or more, LVEF decrease of 10% to a value below 50% or symptomatic heart failure.(122)

Cardiotoxicity rates were similar in patients who were treated in a neoadjuvant or adjuvant manner (13.3% vs. 14.6%). It was highest in patients who received trastuzumab mono-therapy (19.0%) compared to patients who received additional treatment with anthracyclines (13.0%), taxanes (7.1%) or both anthracyclines and taxanes (14.0%). This result was not expected and contradicts other studies in which cardiotoxicity rates have been found to be higher in cases where trastuzumab has been administered concurrently or after anthracycline therapy and anthracycline therapy has been described as a risk factor for the occurrence of trastuzumab-induced cardiotoxicity.(9,70) It might be possible that trastuzumab mono-therapy in our study cohort was administered to patients in which further chemotherapy was contraindicated due to pre-existing co-morbidities, meaning that this sub-group was already at higher risk for developing cardiotoxicity. This could be an explanation for the differing results of our study. However, this theory cannot be proven Our result might also only be a chance effect due to the fairly low number of patients who received trastuzumab mono-therapy in our study.

4.2 Differences between both groups

4.2.1 Co-Morbidity and Risk Factors

An important aspect of our study was the comparison between patients who experienced cardiotoxicity and those who did not regarding co-morbidities and co-therapy. We tested if differences regarding age, BMI, baseline LVEF and arterial hypertension were present in our cohort based on their classification as risk factors.(34,39,70,73,83) Patients who developed cardiotoxicity were older, had a higher BMI, a lower baseline LVEF and were more frequently affected by arterial hypertension than patients without cardiotoxicity. Contrary to other studies our findings did not reach statistical significance, although it

needs to be mentioned that these meta-analyses included far more patients than our retrospective study.(70,73) However, patients with cardiotoxicity did have significantly larger left-ventricular dimensions at baseline (p=0.011). This finding is conclusive to another study published by Okada et al. who found that patients with trastuzumab-induced cardiotoxicity had larger LV end-diastolic dimensions (> or =49 mm) at baseline. They defined cardiotoxicity as LVEF decrease of at least 10% absolute to a value below 55%, which makes it very similar to our study and supports our findings.(123) For diabetes it is not clear whether it is associated with higher rates of trastuzumab-induced cardiotoxicity. Diabetes is not considered a risk factor according to a review by Domercant et al., although they state that smaller studies suggest an association in elderly women with diabetes.(83) Other authors define diabetes as a risk factor in trastuzumab-induced cardiotoxicity.(70,124) In our study population the rate of diabetes was similar in both groups. Different from expectations, smoking was less frequent in the cardiotoxicity group. In other studies there were similar frequencies among patients with and without cardiotoxicity.(37,120) This indicates that smoking habits are not associated with LVEF dysfunction caused by trastuzumab. The difference in our study group is presumably caused by inaccurate data collection rather than a protective effect of smoking habits. Rates for total cholesterol above 200 mg/dl were similar between both groups indicating that there is no association with cardiotoxicity. One has to keep in mind though, that laboratory values were only available in about half of the patients of our study cohort precluding conclusive analyses. None of the observations regarding differences in co-morbid disease among both groups reached statistical significance.

4.2.2 Co-Therapy

Patients who developed cardiotoxicity had a significantly higher rate of ACE-inhibitor medication (p<0.049). This was surprising to some extent, since an aim of this study was to identify possible preventive effects of cardiovascular drugs. These have been described by Wittayanukorn et al. who showed favorable effects of ACEIs/BBs exposition on cardiotoxicity in breast cancer patients treated with anthracyclines and trastuzumab.(111) Also the Manticore trial showed preventive effects on LVEF decrease for the ACEI perindopril and the BB bisoprolol, although effects of bisoprolol were more relevant than those of perindopril.(10) Similarly, findings that were presented by Gujral et al. demonstrated that only prophylactic use of BBs but not ACEIs reduced the extent of LVEF decrease.(125) In summary, there is no consensus on use of ACE-inhibitors in prevention

of trastuzumab-induced cardiotoxicity. On the other hand, it seems plausible that ACE-inhibitors are more common in the cardiotoxicity group since the prevalence of arterial hypertension is also higher in this group. Our results are based on patients who use cardiovascular medication because of a medical condition, which is a completely different setting than patients who take this medication in a preventive manner. This thought can be supported by Yu et al. who found that pre-existing treatment with ACE-inhibitors, beta blockers or angiotensin II receptor antagonists prior to chemotherapy was more common in patients with discontinuation of trastuzumab therapy due to cardiotoxic effects than in patients who received full therapy.(120) Our findings are in accordance with this regarding ACEIs, however, rates for ARBs and beta blockers were similar between both groups in our study cohort, with BBs being used slightly more in the cardiotoxicity group. Also, calcium channel blockers were more common in the cardiotoxicity group. Since they represent another drug commonly used in treatment of arterial hypertension (116) this observation can be attributed to the higher incidence of hypertension in the cardiotoxicity group as well. It needs to be emphasized that apart from ACEIs differences between both groups regarding any other cardiac medication did not reach statistical significance. Statins were more common in the group without cardiotoxicity. It has been demonstrated that statins could prevent LVEF decrease in patients receiving anthracyclines.(126,127) Hence one could assume that statins also protected against major LVEF decrease in our study patients receiving trastuzumab, however no further conclusive data has been published on statin effects on trastuzumab therapy so far. Rates for HCT were similar among patients with and without cardiotoxicity and loop diuretics were slightly more common in the group without cardiotoxicity. It has been demonstrated that spironolactone might protect from anthracycline-induced cardiotoxicity.(128) Based on findings that the aldosterone-bound mineralocorticoid receptor stimulates the EGFR promoter and thus increases EGFR protein levels, Yavas et al. hypothesized that the aldosterone antagonist spironolactone might be effective in trastuzumab-induced cardiotoxicity as well.(129) They could show some protective effects on cardiac tissue in rats who were treated with concomitant trastuzumab therapy and radiation.(130) In our cohort slightly more patients in the group without cardiotoxicity used spironolactone, which could support this thesis. However, the difference was not significant and the number of patients who used this drug was fairly small which prevents us from drawing any conclusions in this case at all. There was only one patient with documented nitrate medication who did not develop cardiotoxicity. The percentage of women with diabetes medication was equal in both groups, which is

conclusive to the fact that diabetes prevalence was also similar in patients with and without cardiotoxicity. Since we included any type of diabetes medication into this group, it would not be reasonable to draw any conclusions on possible preventive effects regarding this group. Aside from ACE-inhibitor medication no significant difference between expected and observed counts could be seen for any of the drugs described above.

4.3 Strengths and Limitations

The strength of this study lies in the relatively large study cohort considering its mono-centric nature with 202 patients included over a fairly long time period. Still it must be kept in mind that cardiotoxicity per se as well as some risk factors and medication were only present in a small number of patients. In addition, the heterogeneity of clinical cohorts is substantial. These factors limit the statistical power of our retrospective study and thus the results are often of descriptive nature and statistical tests need to be interpreted keeping these limitations in mind.

The major limitation of this study is its retrospective nature. Only information that has been collected previously could be used for analysis. Since echocardiographies were performed at different institutions there was no standardized protocol regarding the parameters which were documented or standardized technical equipment. A numerical value of the LVEF was not available in all cases and parameters for left ventricular size varied, if they were available at all. In some cases, the exact results of the echocardiographies were not documented and in some cases it was not clear whether regular echocardiographic follow-up was carried out as recommended. Complete data on co-medication and pre-existing medical conditions was not available in all cases and accuracy of documentation varied among the cases where data was available. It is striking that prevalence rates for obesity and hypercholesterolemia differ among the results extracted from medical records and those derived from BMI and blood analysis. Also, a rather large portion of data was marked as not available. Hence it cannot be precluded that missing data in one of the groups biased our analysis.

Another important difference between our retrospective study and the larger randomized clinical trials are inclusion and exclusion criteria. We basically included all patients irrespective of pre-existing diseases or co-therapy. Also, all patients who were exposed to cardiovascular medication in our case had a prescription because of a medical indication, meaning an already existing risk factor in many cases. Contrary to that the OVERCOME

and MANTICORE trial excluded patients with history of heart failure or relevant cardiac diseases, uncontrolled hypertension and specifically those with prior medication. In that respect our study more closely matches clinical routine and this is also the likely cause of higher observed cardiotoxicity rates.

Because cardiac biomarkers were not analyzed as a routine parameter, they were only available in a small number of patients. Therefore, a possible effect of trastuzumab treatment on cardiac biomarkers could not be assessed in our study cohort. However, our study identified patients suffering from cardiotoxicity and thus laid the base for further analyses of bio-banked materials to measure established biomarkers and identify novel ones.

4.4 Conclusion

Cardiotoxic side effects led to discontinuation of trastuzumab therapy in 11.9% of our study population. Analyzing the change of LVEF, cardiotoxicity was present in 12.4% of patients and up to 25% were affected by milder forms of left ventricular dysfunction. In our study population patients who developed cardiotoxicity had significantly higher baseline LV end-diastolic dimensions and were on ACE-inhibitor medication significantly more often. Furthermore, older age, higher BMI, lower baseline LVEF and higher frequency of arterial hypertension could be observed in the cardiotoxicity group although they failed to reach statistical significance. Diabetes rates were similar among both groups. No conclusive results were found for preventive effects of cardiovascular medication. While ACE inhibitors and calcium channel blockers were more frequent in the cardiotoxicity group, rates for statins and spironolactone were higher in the cardiotoxicity group. Further research is necessary to gain more knowledge on how to prevent trastuzumab-induced cardiotoxicity in prospective clinical trials. This offers the opportunity to evaluate preventive effects of co-medication in drug-naïve patients and facilitates standardized data collection of pre-determined parameters such as echocardiographic indices or cardiac biomarkers.

5 Bibliography

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012: Globocan 2012. Int J Cancer. 2015 Mar 1;136(5):E359–86.
2. Female Breast Cancer - Cancer Stat Facts [Internet]. [cited 2018 Feb 2]. Available from: https://seer.cancer.gov/statfacts/html/breast.html
3. Berry DA, Cronin KA, Plevritis SK, Fryback DG, Clarke L, Zelen M, et al. Effect of Screening and Adjuvant Therapy on Mortality from Breast Cancer. N Engl J Med. 2005 Oct 27;353(17):1784–92.
4. Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, et al. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin. 2016 Jul 1;66(4):271–89.
5. Bradshaw PT, Stevens J, Khankari N, Teitelbaum SL, Neugut AI, Gammon MD. Cardiovascular Disease Mortality Among Breast Cancer Survivors. Epidemiol Camb Mass. 2016 Jan;27(1):6–13.
6. Schairer C, Mink PJ, Carroll L, Devesa SS. Probabilities of Death From Breast Cancer and Other Causes Among Female Breast Cancer Patients. JNCI J Natl Cancer Inst. 2004 Sep 1;96(17):1311–21.
7. Slamon D, Clark G, Wong S, Levin W, Ullrich A, McGuire W. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987 Jan 9;235(4785):177–82.
8. Cronin KA, Harlan LC, Dodd KW, Abrams JS, Ballard-Barbash R. Population-based Estimate of the Prevalence of HER-2 Positive Breast Cancer Tumors for Early Stage Patients in the US. Cancer Invest. 2010 Nov;28(9):963–8.
9. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001;344(11):783–792.
10. Pituskin E, Mackey JR, Koshman S, Jassal D, Pitz M, Haykowsky MJ, et al. Multidisciplinary Approach to Novel Therapies in Cardio-Oncology Research (MANTICORE 101–Breast): A Randomized Trial for the Prevention of Trastuzumab-Associated Cardiotoxicity. J Clin Oncol. 2017 Mar 10;35(8):870–7.
11. Brust - Inzidenz [Internet]. Statistik Austria. [cited 2018 Feb 1]. Available from: http://statistik.at/web_de/statistiken/menschen_und_gesellschaft/gesundheit/krebserkrankungen/brust/index.html
12. Mammakarzinom der Frau — Onkopedia [Internet]. [cited 2017 Nov 7]. Available from: https://www.onkopedia.com/de/onkopedia/guidelines/mammakarzinom-der-frau
13. Brust - Relatives Überleben [Internet]. [cited 2018 Feb 1]. Available from: http://statistik.at/web_de/statistiken/menschen_und_gesellschaft/gesundheit/krebserkrankungen/brust/080572.html
14. Brust - Grafik [Internet]. [cited 2018 Feb 1]. Available from: http://statistik.at/web_de/statistiken/menschen_und_gesellschaft/gesundheit/krebserkrankungen/brust/index.html
15. Sun Y-S, Zhao Z, Yang Z-N, Xu F, Lu H-J, Zhu Z-Y, et al. Risk Factors and Preventions of Breast Cancer. Int J Biol Sci. 2017;13(11):1387.
16. WHO classification of breast tumors [Internet]. [cited 2018 Feb 1]. Available from: http://www.pathologyoutlines.com/topic/breastmalignantwhoclassification.html
17. Lebeau A, Kriegsmann M, Burandt E, Sinn H-P. Invasive Mammakarzinome: Die aktuelle WHO-Klassifikation. Pathol. 2014 Feb;35(1):7–17.

18. Sinn H-P, Kreipe H. A Brief Overview of the WHO Classification of Breast Tumors, 4th Edition, Focusing on Issues and Updates from the 3rd Edition. Breast Care. 2013;8(2):149–54.
19. Wittekind C, Union for International Cancer Control, editors. TNM - Klassifikation maligner Tumoren. Achte Auflage. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA; 2017. 313 p.
20. Tumor Grade [Internet]. National Cancer Institute. [cited 2018 Feb 27]. Available from: https://www.cancer.gov/about-cancer/diagnosis-staging/prognosis/tumor-grade-fact-sheet
21. Mammakarzinom der Frau — Onkopedia [Internet]. [cited 2018 Feb 1]. Available from: https://www.onkopedia.com/de/onkopedia/guidelines/mammakarzinom-der-frau
22. Goldhirsch A, Ingle JN, Gelber RD, Coates AS, Thürlimann B, Senn H-J, et al. Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2009. Ann Oncol. 2009 Aug;20(8):1319.
23. Senkus E, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rutgers E, et al. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015 Sep;26(suppl 5):v8–30.
24. Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. Arch Pathol Lab Med. 2014 Feb;138(2):241–56.
25. S3-Leitlinie Früherkennung, Diagnose, Therapie und Nachsorge des Mammakarzinoms, Kurzversion 4.0, 2017 [Internet]. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF); [cited 2018 Mar 2]. Available from: http://www.leitlinienprogramm - onkologie.de/leitlinien/mammakarzinom/
26. Mammakarzinom der Frau — Onkopedia (04/18) [Internet]. [cited 2018 Apr 24]. Available from: https://www.onkopedia.com/de/onkopedia/guidelines/mammakarzinom-der-frau
27. Teshome M, Hunt KK. Neoadjuvant Therapy in the Treatment of Breast Cancer. Surg Oncol Clin N Am. 2014 Jul;23(3):505–23.
28. Untch M, Konecny GE, Paepke S, von Minckwitz G. Current and future role of neoadjuvant therapy for breast cancer. The Breast. 2014 Oct;23(5):526–37.
29. NCI Dictionary of Cancer Terms. Neoadjuvant therapy [Internet]. National Cancer Institute. [cited 2018 Apr 24]. Available from: https://www.cancer.gov/publications/dictionaries/cancer-terms
30. NCI Dictionary of Cancer Terms. Adjuvant therapy [Internet]. National Cancer Institute. [cited 2018 Apr 24]. Available from: https://www.cancer.gov/publications/dictionaries/cancer-terms
31. Chew HK. Adjuvant therapy for breast cancer: who should get what? West J Med. 2001 Apr;174(4):284–7.
32. NCI Dictionary of Cancer Terms [Internet]. National Cancer Institute. [cited 2017 Oct 5]. Available from: https://www.cancer.gov/publications/dictionaries/cancer-terms
33. Yeh ETH, Bickford CL. Cardiovascular Complications of Cancer Therapy. J Am Coll Cardiol. 2009 Jun;53(24):2231–47.
34. Zamorano JL, Lancellotti P, Rodriguez Muñoz D, Aboyans V, Asteggiano R, Galderisi M, et al. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). Eur Heart J. 2016 Sep 21;37(36):2768–801.
35. Lambert J, Paaladinesh T. Controversies in the Definition of Cardiotoxicity: Do We Care? [Internet]. American College of Cardiology. [cited 2017 Dec 5]. Available from:

http%3a%2f%2fwww.acc.org%2flatest-in-cardiology%2farticles%2f2016%2f07%2f07%2f14%2f59%2fcontroversies-in-the-definition-of-cardiotoxicity
36. Plana JC, Galderisi M, Barac A, Ewer MS, Ky B, Scherrer-Crosbie M, et al. Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: a report from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J - Cardiovasc Imaging. 2014 Oct 1;15(10):1063–93.
37. Suter TM, Procter M, van Veldhuisen DJ, Muscholl M, Bergh J, Carlomagno C, et al. Trastuzumab-Associated Cardiac Adverse Effects in the Herceptin Adjuvant Trial. J Clin Oncol. 2007 Sep;25(25):3859–65.
38. Ewer MS, Ewer SM. Cardiotoxicity of anticancer treatments. Nat Rev Cardiol. 2015 Aug 20;12(11):nrcardio.2015.133.
39. Suter TM, Ewer MS. Cancer drugs and the heart: importance and management. Eur Heart J. 2013 Apr 1;34(15):1102–11.
40. Ewer MS, Lippman SM. Type II Chemotherapy-Related Cardiac Dysfunction: Time to Recognize a New Entity. J Clin Oncol. 2005 May;23(13):2900–2.
41. Telli ML, Hunt SA, Carlson RW, Guardino AE. Trastuzumab-Related Cardiotoxicity: Calling Into Question the Concept of Reversibility. J Clin Oncol. 2007 Aug 10;25(23):3525–33.
42. Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, et al. Early Detection of Anthracycline Cardiotoxicity and Improvement With Heart Failure Therapy. Circulation. 2015 Jun 2;131(22):1981–8.
43. Pentassuglia L, Graf M, Lane H, Kuramochi Y, Cote G, Timolati F, et al. Inhibition of ErbB2 by receptor tyrosine kinase inhibitors causes myofibrillar structural damage without cell death in adult rat cardiomyocytes. Exp Cell Res. 2009 Apr;315(7):1302–12.
44. ElZarrad MK, Mukhopadhyay P, Mohan N, Hao E, Dokmanovic M, Hirsch DS, et al. Trastuzumab Alters the Expression of Genes Essential for Cardiac Function and Induces Ultrastructural Changes of Cardiomyocytes in Mice. Peng T, editor. PLoS ONE. 2013 Nov 8;8(11):e79543.
45. Normanno N, De Luca A, Bianco C, Strizzi L, Mancino M, Maiello MR, et al. Epidermal growth factor receptor (EGFR) signaling in cancer. Gene. 2006 Jan;366(1):2–16.
46. Yarden Y. The EGFR family and its ligands in human cancer: signalling mechanisms and therapeutic opportunities. Eur J Cancer. 2001;37:3–8.
47. Olayioye MA, Neve RM, Lane HA, Hynes NE. NEW EMBO MEMBERS' REVIEW: The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J. 2000 Jul 3;19(13):3159.
48. Yarden Y. Biology of HER2 and its importance in breast cancer. Oncology. 2001;61(Suppl. 2):1–13.
49. Lee K-F, Simon H, Chen H, Bates B, Hung M-C, Hauser C. Requirement for neuregulin receptor erbB2 in neural and cardiac development. Nature. 1995 Nov;378(6555):394–8.
50. Vermeulen Z, Segers VFM, De Keulenaer GW. ErbB2 signaling at the crossing between heart failure and cancer. Basic Res Cardiol [Internet]. 2016 Nov [cited 2017 Oct 7];111(6). Available from: http://link.springer.com/10.1007/s00395-016-0576-z
51. Brustkrebs: EU-Zulassung für Trastuzumab [Internet]. DAZ.online. 2000 [cited 2017 Oct 13]. Available from: https://www.deutsche-apotheker-zeitung.de/daz-az/2000/daz-37-2000/uid-7226
52. Hudis CA. Trastuzumab—mechanism of action and use in clinical practice. N Engl J Med. 2007;357(1):39–51.

53. Rochette L, Guenancia C, Gudjoncik A, Hachet O, Zeller M, Cottin Y, et al. Anthracyclines/trastuzumab: new aspects of cardiotoxicity and molecular mechanisms. Trends Pharmacol Sci. 2015 Jun;36(6):326–48.
54. Albanell J, Bellmunt J, Molina R, García M, Caragol I, Bermejo B, et al. Node-negative breast cancers with p53(-)/HER2-neu(-) status may identify women with very good prognosis. Anticancer Res. 1996 Apr;16(2):1027–32.
55. European Medicines Agency - Find medicine - Herceptin [Internet]. [cited 2017 Oct 12]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000278/human_med_000818.jsp&mid=WC0b01ac058001d124#product-info
56. Ismael G, Hegg R, Muehlbauer S, Heinzmann D, Lum B, Kim S-B, et al. Subcutaneous versus intravenous administration of (neo) adjuvant trastuzumab in patients with HER2-positive, clinical stage I–III breast cancer (HannaH study): a phase 3, open-label, multicentre, randomised trial. Lancet Oncol. 2012;13(9):869–878.
57. Pivot X, Verma S, Fallowfield L, Müller V, Lichinitser M, Jenkins V, et al. Efficacy and safety of subcutaneous trastuzumab and intravenous trastuzumab as part of adjuvant therapy for HER2-positive early breast cancer: Final analysis of the randomised, two-cohort PrefHer study. Eur J Cancer. 2017 Nov;86:82–90.
58. İçli F, Altundağ K, Akbulut H, Paydaş S, Başaran G, Saip P, et al. Nine weeks versus 1 year adjuvant trastuzumab in patients with early breast cancer: an observational study by the Turkish Oncology Group (TOG). Breast Cancer. 2015 Sep;22(5):480–5.
59. Mantarro S, Rossi M, Bonifazi M, D'Amico R, Blandizzi C, La Vecchia C, et al. Risk of severe cardiotoxicity following treatment with trastuzumab: a meta-analysis of randomized and cohort studies of 29,000 women with breast cancer. Intern Emerg Med. 2016 Feb;11(1):123–40.
60. Valabrega G, Montemurro F, Aglietta M. Trastuzumab: mechanism of action, resistance and future perspectives in HER2-overexpressing breast cancer. Ann Oncol. 2007 Apr 10;18(6):977–84.
61. Albini A, Cesana E, Donatelli F, Cammarota R, Bucci EO, Baravelli M, et al. Cardio-oncology in targeting the HER receptor family: the puzzle of different cardiotoxicities of HER2 inhibitors. Future Cardiol. 2011 Sep;7(5):693–704.
62. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. Nat Med. 2000 Apr 1;6(4):nm0400_443.
63. Christianson TA, Doherty JK, Lin YJ, Ramsey EE, Holmes R, Keenan EJ, et al. NH2-terminally Truncated HER-2/neu Protein: Relationship with Shedding of the Extracellular Domain and with Prognostic Factors in Breast Cancer. Cancer Res. 1998 Nov 15;58(22):5123–9.
64. Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J. Trastuzumab (Herceptin), a Humanized Anti-HER2 Receptor Monoclonal Antibody, Inhibits Basal and Activated HER2 Ectodomain Cleavage in Breast Cancer Cells. Cancer Res. 2001 Jun 15;61(12):4744–9.
65. Köstler WJ, Schwab B, Singer CF, Neumann R, Rücklinger E, Brodowicz T, et al. Monitoring of Serum Her-2/neu Predicts Response and Progression-Free Survival to Trastuzumab-Based Treatment in Patients with Metastatic Breast Cancer. Clin Cancer Res. 2004 Mar 1;10(5):1618–24.
66. Heregulin selectively upregulates vascular endothelial growth factor secretion in cancer cells and stimulates angiogenesis. Publ Online 21 July 2000 Doi101038sjonc1203685 [Internet]. 2000 Jul 21 [cited 2017 Nov 8];19(31). Available from: http://www-1nature-1com-1pubmed.han.medunigraz.at/onc/journal/v19/n31/full/1203685a.html

67. FDA Approval for Trastuzumab [Internet]. National Cancer Institute. [cited 2017 Oct 13]. Available from: https://www.cancer.gov/about-cancer/treatment/drugs/fda-trastuzumab
68. Dang CT, Yu AF, Jones LW, Liu J, Steingart RM, Argolo DF, et al. Cardiac surveillance guidelines for trastuzumab-containing therapy in early-stage breast cancer: getting to the heart of the matter. J Clin Oncol. 2016;34(10):1030–1033.
69. Fachinformation-Herceptin-150-mg-032017.pdf.
70. Jawa Z, Perez RM, Garlie L, Singh M, Qamar R, Khandheria BK, et al. Risk factors of trastuzumab-induced cardiotoxicity in breast cancer: A meta-analysis. Medicine (Baltimore). 2016 Nov;95(44):e5195.
71. Brana I, Tabernero J. Cardiotoxicity. Ann Oncol. 2010 Oct 1;21(Supplement 7):vii173-vii179.
72. Gunaldi M, Duman BB, Afsar CU, Paydas S, Erkisi M, Kara IO, et al. Risk factors for developing cardiotoxicity of trastuzumab in breast cancer patients: An observational single-centre study. J Oncol Pharm Pract [Internet]. 2015 Jan 7 [cited 2017 Oct 13]; Available from: http://journals-1sagepub-1com-1pubmed.han.medunigraz.at/doi/abs/10.1177/1078155214567162?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed
73. Guenancia C, Lefebvre A, Cardinale D, Yu AF, Ladoire S, Ghiringhelli F, et al. Obesity As a Risk Factor for Anthracyclines and Trastuzumab Cardiotoxicity in Breast Cancer: A Systematic Review and Meta-Analysis. J Clin Oncol. 2016 Sep 10;34(26):3157–65.
74. Force T, Krause DS, Van Etten RA. Molecular mechanisms of cardiotoxicity of tyrosine kinase inhibition. Nat Rev Cancer. 2007 May;7(5):332–44.
75. Necela BM, Axenfeld BC, Serie DJ, Kachergus JM, Perez EA, Thompson EA, et al. The antineoplastic drug, trastuzumab, dysregulates metabolism in iPSC-derived cardiomyocytes. Clin Transl Med [Internet]. 2017 Dec [cited 2017 Oct 23];6(1). Available from: http://clintransmed.springeropen.com/articles/10.1186/s40169-016-0133-2
76. Mohan N, Shen Y, Endo Y, ElZarrad MK, Wu WJ. Trastuzumab, but Not Pertuzumab, Dysregulates HER2 Signaling to Mediate Inhibition of Autophagy and Increase in Reactive Oxygen Species Production in Human Cardiomyocytes. Mol Cancer Ther. 2016 Jun 1;15(6):1321–31.
77. Hedhli N, S Russell K. Cardiotoxicity of molecularly targeted agents. Curr Cardiol Rev. 2011;7(4):221–233.
78. Sendur MAN, Aksoy S, Altundag K. Cardiotoxicity of novel HER2-targeted therapies. Curr Med Res Opin. 2013 Aug 1;29(8):1015–24.
79. Choi HD, Chang MJ. Cardiac toxicities of lapatinib in patients with breast cancer and other HER2-positive cancers: a meta-analysis. Breast Cancer Res Treat [Internet]. 2017 Aug 19 [cited 2017 Oct 24]; Available from: http://link.springer.com/10.1007/s10549-017-4460-9
80. Grazette LP, Boecker W, Matsui T, Semigran M, Force TL, Hajjar RJ, et al. Inhibition of ErbB2 causes mitochondrial dysfunction in cardiomyocytes. J Am Coll Cardiol. 2004 Dec;44(11):2231–8.
81. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011 Jan 13;469(7329):221–5.
82. Mutschler E, Geisslinger G, Kroemer HK, Menzel S, Ruth P, editors. Mutschler Arzneimittelwirkungen: Lehrbuch der Pharmakologie, der klinischen Pharmakologie und Toxikologie ; mit einführenden Kapiteln in die Anatomie, Physiologie und Pathophysiologie. 10., vollständig überarbeitete und erweiterte Auflage. Stuttgart: WVG, Wissenschaftliche Verlagsgesellschaft; 2013. 1197 p.

83. Domercant J, Polin N, Jahangir E. Cardio-Oncology: A Focused Review of Anthracycline-, Human Epidermal Growth Factor Receptor 2 Inhibitor–, and Radiation-Induced Cardiotoxicity and Management. Ochsner J. 2016;16(3):250–256.
84. McGowan JV, Chung R, Maulik A, Piotrowska I, Walker JM, Yellon DM. Anthracycline Chemotherapy and Cardiotoxicity. Cardiovasc Drugs Ther. 2017 Feb;31(1):63–75.
85. Mele D, Nardozza M, Spallarossa P, Frassoldati A, Tocchetti CG, Cadeddu C, et al. Current views on anthracycline cardiotoxicity. Heart Fail Rev. 2016 Sep;21(5):621–34.
86. Sadurska E. Current Views on Anthracycline Cardiotoxicity in Childhood Cancer Survivors. Pediatr Cardiol. 2015 Aug;36(6):1112–9.
87. Lyu YL, Kerrigan JE, Lin C-P, Azarova AM, Tsai Y-C, Ban Y, et al. Topoisomerase II Mediated DNA Double-Strand Breaks: Implications in Doxorubicin Cardiotoxicity and Prevention by Dexrazoxane. Cancer Res. 2007 Sep 15;67(18):8839–46.
88. Zhang S, Liu X, Bawa-Khalfe T, Lu L-S, Lyu YL, Liu LF, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nat Med. 2012 Oct 28;18(11):1639–42.
89. Zhao L, Zhang B. Doxorubicin induces cardiotoxicity through upregulation of death receptors mediated apoptosis in cardiomyocytes. Sci Rep. 2017 Mar 16;7:44735.
90. de Korte MA, de Vries EGE, Lub-de Hooge MN, Jager PL, Gietema JA, van der Graaf WTA, et al. 111Indium-trastuzumab visualises myocardial human epidermal growth factor receptor 2 expression shortly after anthracycline treatment but not during heart failure: A clue to uncover the mechanisms of trastuzumab-related cardiotoxicity. Eur J Cancer. 2007 Sep;43(14):2046–51.
91. Curigliano G, Cardinale D, Dent S, Criscitiello C, Aseyev O, Lenihan D, et al. Cardiotoxicity of anticancer treatments: Epidemiology, detection, and management. CA Cancer J Clin. 2016 Jul 1;66(4):309–25.
92. Ewer MS, Ali MK, Mackay B, Wallace S, Valdivieso M, Legha SS, et al. A comparison of cardiac biopsy grades and ejection fraction estimations in patients receiving Adriamycin. J Clin Oncol. 1984;2(2):112–117.
93. Ewer MS, Lenihan DJ. Left ventricular ejection fraction and cardiotoxicity: is our ear really to the ground? American Society of Clinical Oncology; 2008.
94. Sawaya H, Sebag IA, Plana JC, Januzzi JL, Ky B, Tan TC, et al. Assessment of Echocardiography and Biomarkers for the Extended Prediction of Cardiotoxicity in Patients Treated With Anthracyclines, Taxanes, and Trastuzumab. Circ Cardiovasc Imaging. 2012 Sep 1;5(5):596–603.
95. Gorcsan J, Tanaka H. Echocardiographic Assessment of Myocardial Strain. J Am Coll Cardiol. 2011 Sep;58(14):1401–13.
96. Lipshultz SE, Rifai N, Sallan SE, Lipsitz SR, Dalton V, Sacks DB, et al. Predictive Value of Cardiac Troponin T in Pediatric Patients at Risk for Myocardial Injury. Circulation. 1997 Oct 21;96(8):2641–8.
97. Lipshultz SE, Miller TL, Scully RE, Lipsitz SR, Rifai N, Silverman LB, et al. Changes in Cardiac Biomarkers During Doxorubicin Treatment of Pediatric Patients With High-Risk Acute Lymphoblastic Leukemia: Associations With Long-Term Echocardiographic Outcomes. J Clin Oncol. 2012 Apr 1;30(10):1042.
98. Cardinale D, Sandri MT, Martinoni A, LabTech AT, Civelli M, Lamantia G, et al. Left ventricular dysfunction predicted by early troponin I release after high-dose chemotherapy. J Am Coll Cardiol. 2000;36(2):517–522.
99. Cardinale D, Sandri MT, Colombo A, Colombo N, Boeri M, Lamantia G, et al. Prognostic Value of Troponin I in Cardiac Risk Stratification of Cancer Patients Undergoing High-Dose Chemotherapy. Circulation. 2004 Jun 8;109(22):2749–54.

100. Cardinale D, Colombo A, Torrisi R, Sandri MT, Civelli M, Salvatici M, et al. Trastuzumab-induced cardiotoxicity: clinical and prognostic implications of troponin I evaluation. J Clin Oncol. 2010;28(25):3910–3916.
101. Stevens PL, Lenihan DJ, Ky B, Warneke CL, Johnson MM, Abramson VG, et al. Prediction and early detection of anthracycline-related cardiotoxicity using cardiac biomarkers. J Clin Oncol. 2014 May 20;32(15_suppl):9644–9644.
102. Ky B, Warneke CL, Lenihan DJ, Cheema PS, Moore DF, Campbell MG, et al. Clinical risk prediction in anthracycline cardiotoxicity. J Clin Oncol. 2014 May 20;32(15_suppl):9624–9624.
103. Sandri MT. N-Terminal Pro-B-Type Natriuretic Peptide after High-Dose Chemotherapy: A Marker Predictive of Cardiac Dysfunction? Clin Chem. 2005 Aug 1;51(8):1405–10.
104. Stevens PL, Lenihan DJ. Cardiotoxicity due to Chemotherapy: the Role of Biomarkers. Curr Cardiol Rep. 2015 Jul 1;17(7):49.
105. Utkan G, Yalcin B, Akbulut H, Onur H, Oztuna DG, Demirkazk A, et al. The role of cardiac biomarkers as predictors of trastuzumab cardiotoxicity in patients with breast cancer. Exp Oncol. 2015;(37,№ 1):53–57.
106. Fallah-Rad N, Walker JR, Wassef A, Lytwyn M, Bohonis S, Fang T, et al. The Utility of Cardiac Biomarkers, Tissue Velocity and Strain Imaging, and Cardiac Magnetic Resonance Imaging in Predicting Early Left Ventricular Dysfunction in Patients With Human Epidermal Growth Factor Receptor II–Positive Breast Cancer Treated With Adjuvant Trastuzumab Therapy. J Am Coll Cardiol. 2011 May;57(22):2263–70.
107. Ky B, Putt M, Sawaya H, French B, Januzzi JL, Sebag IA, et al. Early Increases in Multiple Biomarkers Predict Subsequent Cardiotoxicity in Patients With Breast Cancer Treated With Doxorubicin, Taxanes, and Trastuzumab. J Am Coll Cardiol. 2014 Mar;63(8):809–16.
108. Putt M, Hahn VS, Januzzi JL, Sawaya H, Sebag IA, Plana JC, et al. Longitudinal Changes in Multiple Biomarkers Are Associated with Cardiotoxicity in Breast Cancer Patients Treated with Doxorubicin, Taxanes, and Trastuzumab. Clin Chem. 2015 Sep 1;61(9):1164–72.
109. Gulati G, Heck SL, Ree AH, Hoffmann P, Schulz-Menger J, Fagerland MW, et al. Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 × 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol. Eur Heart J. 2016 Jun 1;37(21):1671–80.
110. Bosch X, Rovira M, Sitges M, Domènech A, Ortiz-Pérez JT, de Caralt TM, et al. Enalapril and Carvedilol for Preventing Chemotherapy-Induced Left Ventricular Systolic Dysfunction in Patients With Malignant Hemopathies. J Am Coll Cardiol. 2013 Jun;61(23):2355–62.
111. Wittayanukorn S, Qian J, Westrick SC, Billor N, Johnson B, Hansen RA. Prevention of Trastuzumab and Anthracycline-induced Cardiotoxicity Using Angiotensin-converting Enzyme Inhibitors or β-blockers in Older Adults With Breast Cancer. Am J Clin Oncol [Internet]. 2017 May 23 [cited 2017 Nov 18];Publish Ahead of Print. Available from: https://insights.ovid.com/pubmed?pmid=28537988
112. Boekhout AH, Gietema JA, Kerklaan BM, Werkhoven ED van, Altena R, Honkoop A, et al. Angiotensin II–Receptor Inhibition With Candesartan to Prevent Trastuzumab-Related Cardiotoxic Effects in Patients With Early Breast Cancer: A Randomized Clinical Trial. JAMA Oncol. 2016 Aug 1;2(8):1030–7.
113. Prevention of Anthracycline-induced Cardiotoxicity - Full Text View - ClinicalTrials.gov [Internet]. [cited 2017 Nov 18]. Available from: https://clinicaltrials.gov/ct2/show/NCT01968200

114. Meattini I, Curigliano G, Terziani F, Becherini C, Airoldi M, Allegrini G, et al. SAFE trial: an ongoing randomized clinical study to assess the role of cardiotoxicity prevention in breast cancer patients treated with anthracyclines with or without trastuzumab. Med Oncol. 2017 May 1;34(5):75.
115. Guglin M, Munster P, Fink A, Krischer J. Lisinopril or Coreg CR in reducing cardiotoxicity in women with breast cancer receiving trastuzumab: A rationale and design of a randomized clinical trial. Am Heart J. 2017 Jun;188:87–92.
116. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J. 2016 Jul 14;37(27):2129–200.
117. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J – Cardiovasc Imaging. 2015 Mar;16(3):233–71.
118. Lee CK, Margossian R, Sleeper LA, Canter CE, Chen S, Tani LY, et al. Variability of M-mode versus two-dimensional echocardiography measurements in children with dilated cardiomyopathy. Pediatr Cardiol. 2014 Apr;35(4):658–67.
119. Body mass index - BMI [Internet]. 2018 [cited 2018 Feb 21]. Available from: http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi
120. Yu AF, Yadav NU, Lung BY, Eaton AA, Thaler HT, Hudis CA, et al. Trastuzumab interruption and treatment-induced cardiotoxicity in early HER2-positive breast cancer. Breast Cancer Res Treat. 2015 Jan;149(2):489–95.
121. Tarantini L, Cioffi G, Gori S, Tuccia F, Boccardi L, Bovelli D, et al. Trastuzumab Adjuvant Chemotherapy and Cardiotoxicity in Real-World Women With Breast Cancer. J Card Fail. 2012 Feb 1;18(2):113–9.
122. Piotrowski G, Gawor R, Stasiak A, Gawor Z, Potemski P, Banach M. Cardiac complications associated with trastuzumab in the setting of adjuvant chemotherapy for breast cancer overexpressing human epidermal growth factor receptor type 2 – a prospective study. Arch Med Sci AMS. 2012 May 9;8(2):227.
123. Okada Y, Kanbayashi C, Sato N. [Trastuzumab-associated cardiac dysfunction]. Gan To Kagaku Ryoho. 2010 Apr;37(4):665–9.
124. Serrano C, Cortés J, De Mattos-Arruda L, Bellet M, Gómez P, Saura C, et al. Trastuzumab-related cardiotoxicity in the elderly: a role for cardiovascular risk factors. Ann Oncol. 2012 Apr 1;23(4):897–902.
125. Gujral DM, Lloyd G, Bhattacharyya S. Effect of prophylactic betablocker or ACE inhibitor on cardiac dysfunction & heart failure during anthracycline chemotherapy ± trastuzumab. The Breast. 2018 Feb;37:64–71.
126. Acar Z, Kale A, Turgut M, Demircan S, Durna K, Demir S, et al. Efficiency of Atorvastatin in the Protection of Anthracycline-Induced Cardiomyopathy. J Am Coll Cardiol. 2011 Aug;58(9):988–9.
127. Chotenimitkhun R, D'Agostino R, Lawrence JA, Hamilton CA, Jordan JH, Vasu S, et al. Chronic Statin Administration May Attenuate Early Anthracycline-Associated Declines in Left Ventricular Ejection Function. Can J Cardiol. 2015 Mar;31(3):302–7.
128. Akpek M, Ozdogru I, Sahin O, Inanc M, Dogan A, Yazici C, et al. Protective effects of spironolactone against anthracycline-induced cardiomyopathy: Effects of spironolactone on anthracycline cardiomyopathy. Eur J Heart Fail. 2015 Jan;17(1):81–9.
129. Yavas G, Elsurer R, Yavas C, Elsurer C, Ata O. Does spironolactone ameliorate trastuzumab-induced cardiac toxicity? Med Hypotheses. 2013 Aug;81(2):231–4.

130. Yavas G, Celik E, Yavas C, Elsurer C, Afsar RE. Spironolactone ameliorates the cardiovascular toxicity induced by concomitant trastuzumab and thoracic radiotherapy. Rep Pract Oncol Radiother. 2017 Jul;22(4):295–302.