BIOMARKERS
FOR THE EARLY DETECTION OF
CANCER TREATMENT INDUCED
CARDIOTOXICITY

Ben Bulten

BIOMARKERS FOR THE EARLY DETECTION OF CANCER TREATMENT INDUCED CARDIOTOXICITY

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1

General introduction and outline of the thesis

Derived from:

Autonomic imaging of cardiotoxicity with ^{123}I -mIBG: the effects of chemotherapy, monoclonal antibody therapy and radiotherapy.

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General Introduction

In recent years, the overall five year survival rate of patients with resectable breast cancer (i.e. stage IIB and stage IIIA to C) has increased to 81%, depending on disease stage at diagnosis.^{1,2} Although advances in research have offered new opportunities for tailored treatment, for example in human epidermal receptor 2 (HER2) positive tumours, the mainstay of systemic treatment still is (neo) adjuvant chemotherapy.³ Depending on tumour stage, tumour type and patient characteristics, one can choose from different chemotherapeutic regimens, including taxanes, fluorouracil, cyclophosphamide and anthracyclines.³

Unfortunately, all anti cancer therapeutics may have undesirable effects. Some of the most significant side effects affect the cardiovascular system and are designated as cardiotoxicity. Mostly, cardiotoxic effects are transient, but irreversible cardiac damage does occur in a minority of patients and may cause clinically overt congestive heart failure (CHF). In general, three types of (anthracycline-induced) cardiotoxicity can be distinguished:

- Acute cardiotoxicity: occurs in <1% of patients immediately after infusion and is usually reversible.
- Early-onset chronic progressive cardiotoxicity: occurs in 1.6% to 2.1% of patients during therapy or within one year after treatment.
- Late-onset chronic progressive cardiotoxicity: occurs in 1.6% to 5% of patients at least one year but up to twenty years after treatment.^{4,5}

This thesis will mainly focus on anthracycline-induced cardiotoxicity (AIC). However, in the next section other treatments are also discussed, since these are often given before, after or during anthracycline treatment and therefore potentially aggravate cardiotoxicity.

Cardiotoxic effects of chemotherapy

Many chemotherapeutic agents have a spectrum of cardiotoxic effects (Table 1.1).^{4,6} These effects vary from mild, transient changes in cardiac function during or immediately after treatment to more serious complications at a later stage, which may result in irreversible cardiac dysfunction or CHF.

Anthracyclines

Since their discovery in the 1960s, the class of antineoplastic drugs known as anthracyclines has developed in the most widely used antitumor drugs, displaying the broadest spectrum of antitumor activity known.⁷ Anthracyclines are used against both solid and haematological types of cancer, including leukaemia, lymphoma, lung cancer and breast

Table 1.1 Cardiotoxicity profiles of anti-cancer agents.

Generic drug name	Relative frequency of therapeutic use	Cardiac AE	Relative frequency of AE*
Anthracyclines/ anthraquinolones			
Doxorubicin	Very frequent	CHF/LVD	Common
Daunorubicin	Very Frequent	CHF/LVD	Common
Epirubicin	Very frequent	CHF/LVD	Common
Idarubicin	Very Frequent	CHF/LVD	Common
Mitoxantrone	Infrequent	CHF/LVD	Uncommon
Alkylating agents			
Busulfan	Infrequent	Edomyocardial fibrosis	Rare
	Infrequent	Cardiac tamponade	Rare
Cisplatin	Very frequent	Ischemia	Uncommon
	Very frequent	Hypertension	Frequent
	Very frequent	CHF/LVD	Uncommon
Cyclophosphamide	Very frequent	Pericarditis/myocarditis	Rare
	Very frequent	CHF/LVD	Uncommon
Ifosfamide	Common	CHF/LVD	Uncommon
	Common	Arrhythmia	Uncommon
Mitomycin	Infrequent	CHF/LVD	Uncommon
Antimetabolites			
Capecitabine	Very frequent	Ischemia	Rare
Cytarabine, Ara-C	Very frequent	Pericarditis	Rare
	Very frequent	CHF/LVD	Rare
Fluorouracil	Very frequent	Ischemia	Uncommon
	Very frequent	Cardiogenic shock	Rare
Antimicrotubules			
Paclitaxel	Very frequent	Arrhythmia	Rare
	Very frequent	Hypotension	Rare
	Very frequent	CHF/LVD	Uncommon
Vinca alkaloids	Common	Ischemia	Uncommon

Table 1.1 Continued.

Generic drug name	Relative frequency of therapeutic use	Cardiac AE	Relative frequency of AE*
Monoclonal antibodies			
Alemtuzumab	Infrequent	Hypotension	Common
	Infrequent	CHF/LVD	Rare
Bevacizumab	Common	Hypertension	Common
	Common	CHF/LVD	Uncommon
Cetuximab	Common	Hypotension	Rare
Rituximab	Common	Hypotension	Uncommon
	Common	Arrhythmia	Uncommon
Trastuzumab	Common	CHF/LVD	Uncommon
Interleukins			
IL-2	Infrequent	Hypotension	Frequent
	Infrequent	Arrhythmia	Uncommon
Denileukindiftox	Infrequent	Hypotension	Frequent
Interferon α	Very frequent	Hypotension	Common
	Very frequent	Ischemia	Uncommon
	Very frequent	CHF/LVD	Rare
Miscellaneous			
All-trans retinoic acid	Infrequent	CHF/LVD	Uncommon
	Infrequent	Hypotension	Uncommon
	Infrequent	Pericardial effusion	Rare
Arsenic trioxide	Infrequent	QT prolongation	Frequent
Imatinib	Very Frequent	Pericardial effusion	Uncommon
	Very frequent	CHF/LVD	Common
Pentostatin	Infrequent	CHF/LVD	Uncommon
Thalidomide	Infrequent	Edema	Uncommon
	Infrequent	Hypotension	Rare
	Infrequent	Arrhythmia	Uncommon
Etoposide	Common	Hypotension	Uncommon

AE: adverse events. LVD: left ventricle dysfunction.
Relative incidence of use: as estimated by a tertiary Unites States-based cancer center. Very frequent: >5000 doses per year. Common: 1000-5000 doses per year. Infrequent: <1000 doses per year. Table derived from Yeh et al.⁴
Relative frequency of AE: rare <1%, uncommon 1-5%, common 6-10%, frequent >10%.

cancer. Available agents include daunorubicin, epirubicin and doxorubicin.⁷ The latter two are most often used in the treatment of advanced breast cancer.³ Unfortunately, the powerful antineoplastic effects of anthracyclines come at a prize: use of anthracyclines will lead to overt CHF in 6% of cases.⁸ A meta-analysis of 55 randomized controlled trials demonstrated an increased risk of clinical cardiotoxicity by anthracycline-based regimens by 5.43 fold, subclinical cardiotoxicity by 6.25 fold, and of cardiac death by 4.94 fold compared with non-anthracycline regimens.⁹ Risk factors for developing AIC comprise a high cumulative anthracycline dose, mediastinal radiation therapy, combination chemotherapy, combined chemo- and monoclonal antibody therapy, pre-existing cardiovascular disease, emphysema, diabetes, female sex, and very young or older age.^{4,6}

AIC may occur early (during or immediately after infusion) or late (up to twenty years after therapy). Early toxic effects are usually self-limiting after discontinuation of therapy, while late AIC may persist and typically presents with a dilated cardiomyopathy. Symptoms can range from none to severe cardiac impairment and even death.⁴

Molecular mechanisms

There are several hypotheses on the molecular mechanisms by which doxorubicin (and anthracyclines in general) affects both tumorous and healthy tissue.¹⁰ For a long time, the production of highly reactive oxygen free radicals has been suspected to be the most important process.¹¹ Hence, iron chelation and subsequent reduction of free radicals has been suggested as a protective mechanism.¹² However, the administration of potent iron chelators or antioxidants has not shown any protection against AIC.¹³⁻¹⁵

Therefore, the molecular basis of the antineoplastic effect of anthracyclines is nowadays thought to lie in the binding of the enzyme topoisomerase II (Top2) to DNA, forming the Top2-DNA complex and triggering cell death.^{10, 16} There are two known Top2 enzymes: Top2 α and Top2 β . While Top2 α is over expressed in proliferating cells, it is not in 'normal' tissue. Top2 β , on the other hand, is present in all cells, including cardiomyocytes, although the exact extent of expression is not known.^{17, 18} Anthracyclines target both iso-enzymes.¹⁹ Therefore, the mechanism by which anthracyclines interact with Top2 β is a potential target for the reduction of cardiotoxicity.

Dexrazoxane (also known as Cardioxane) is a cardioprotective drug that has been successfully used in the clinic to reduce AIC.¹⁵ However, evidence is emerging that dexrazoxane might decrease the antineoplastic efficacy of anthracyclines.^{15, 20} While initially the iron-chelating hypothesis was suspected to be the main interaction mechanism of dexrazoxane, nowadays the core molecular interaction seems to be the prevention of the formation of Top2-DNA complexes.^{10, 11, 20} That this effect also targets the over expressed Top2 α on tumorous cells explains the observed decrease in anthracycline efficacy.

Other chemotherapeutics

Two well-known classes of cardiotoxic chemotherapeutics other than anthracyclines, are taxanes (e.g. paclitaxel, docetaxel) and ankyating agents (e.g. cyclophosphamide). These therapies are often used in combination with anthracyclines to treat breast cancer in a regimen called TAC (Taxanes, Adriamycin, Cyclophosphamide). Taxanes may induce bradycardia, myocardial ischemia and CHF. Ankyating agents may cause cardiac inflammation and arrhythmias. However, cardiotoxicity caused by these therapeutics is often mild and reversible.²¹ Nonetheless, when administered in combination with anthracyclines careful monitoring of the cardiac status is advisable.

Cardiotoxic effects of monoclonal antibodies

A relatively new class of anticancer agents are monoclonal antibodies, which are nowadays used against a broad spectrum of cancer types, including melanoma, lymphoma, colorectal cancer and breast cancer. Monoclonal antibodies are engineered to target disease-specific proteins, thereby reducing the chance of side effects. However, trastuzumab, the monoclonal antibody directed against HER2, is well-known for its cardiotoxic potential.

Trastuzumab

Trastuzumab (Herceptin®) is known to cause a (mostly) transient asymptomatic decline in left ventricular ejection fraction (LVEF), but can result in CHF years after therapy. Progress towards CHF is not dose-dependent.²² Trastuzumab-induced cardiotoxicity (TIC) incidence varies according to the definition used, but ranges from 2% to 7% for monotherapy, from 2% to 13% in combination with paclitaxel and up to 27% when combined with anthracyclines.²³ Patients receiving a combined chemotherapeutic regimen including trastuzumab are at highest risk to present a cardiac event, CHF or cardiac death.^{24, 25} In TIC, the main pathophysiologic mechanism is inhibition of HER2 in cardiomyocyte tissue. The HER2-pathway is required for cell survival and continuing function and seems to be stimulated in situations of myocardial stress, such as anthracycline treatment.²⁶ HER2 inhibition results in depletion of adenosine triphosphate (ATP) and subsequent dysfunction of contractility.²⁷

Other monoclonal antibodies

Bevacizumab is a monoclonal antibody that targets vascular endothelial growth factor and is believed to improve the outcome of several malignancies, like advanced breast cancer, colorectal cancer and non-small-cell lung cancer.²⁸ In an extensive meta-analysis in 3784 patients treated with this drug, an overall incidence of 1.6% and a relative risk of 4.7 for CHF was found, although the relevance of these findings is still under debate.^{28, 29} Other potential cardiac side effects are hypertension and myocardial ischemia, although

the latter is rare.⁴ The cardiotoxic profiles of other monoclonal antibodies are listed in table 1.1.

Cardiotoxic effects of radiotherapy

Radiotherapy to the (left side of the) chest may also induce a variety of cardiovascular complications, such as myocardial fibrosis, diastolic dysfunction (DD), pericarditis, coronary artery disease, valvular abnormalities and conduction disturbances.^{30, 31} The patho-physiologic mechanism relies on direct injury from the high-energy radiation beam, leading to diffuse fibrosis and capillary narrowing.³² Once fibrosis has developed, this is irreversible.³³ Risk factors for radiation-induced cardiotoxicity (RIC) include a radiation dose >30 Gray, a fraction dose >2 Gray, a large volume of irradiated heart, younger age, longer time since exposure, the use of concomitant anti cancer therapy and the presence of other cardiovascular risk factors (i.e. diabetes, hypertension, dyslipidemia, obesity or smoking).

Cardiac morbidity and mortality due to RIC was increased in several large, multicenter registries.³⁴ In one study the relative risk of cardiovascular mortality in patients treated with thoracic radiotherapy was 1.27.³⁵ Radiotherapy for left-sided breast cancer induced volume-dependent myocardial perfusion defects with ^{99m}Tc-tetrofosmin or sestamibi scintigraphy in approximately 40% of patients.³⁶ The perfusion defects were associated with corresponding wall motion abnormalities. However, long-term clinical consequences of these findings are not known.

With the development of modern radiotherapy techniques, such as three-dimensional treatment planning, linear accelerator photons or multiple-field conformal or intensity modulation, post-radiation cardiotoxicity has already shown a declining trend.³⁷ However, the irreversibility of the side effects and the increased group of patients that are eligible for radiotherapy still renders RIC a clinically significant complication.

Monitoring the heart function

Because of the potentially severe adverse cardiac events, clinicians monitor the heart function of all patients at baseline and during trastuzumab therapy and patients with increased cardiovascular risk during anthracycline therapy. The current gold standard to evaluate cardiac function in relation to cardiotoxicity is the assessment of LVEF by multi-gated radionuclide ventriculography (MUGA) or echocardiography at rest.³⁸ When the LVEF drops, clinicians can choose to discontinue or postpone the next administration,

reduce the total dose or start prophylactic medication. However, LVEF will only decrease when a certain critical mass of cell damage has occurred. Consequently, at the moment of detection, the impairment in cardiac function cannot be reversed by therapeutic interventions. Above that, these techniques tend to underestimate the actual damage.³⁹ It is therefore obvious that early detection of subclinical cancer treatment induced cardiotoxicity is important: the clinician might reduce the dose of the cardiotoxic therapy and/or start prophylactic medication (i.e. beta blockers, dexrazoxane, ACE inhibitors) before irreversible damage has occurred. This leads to a decrease in clinical cardiotoxicity, thus further improving the life expectancy and quality of life of (breast) cancer survivors. However, an accurate method to identify cardiotoxicity in a subclinical stage ('early detection') has not yet been developed, although many have been proposed.⁴⁰ In the next section the different methods that are evaluated in the current thesis are briefly introduced.

¹²³I-mIBG scintigraphy

Meta-iodenbenzylguanidine (mIBG) is a guanethidine analog, which is taken up, concentrated and stored in the presynaptic nerve terminals of the sympathetic nervous system in a manner similar to norepinephrine (NE).^{41, 42} In contrast with NE, mIBG is not catabolised, but retained in the sympathetic nerve endings after uptake (Figure 1.1). Labelling of mIBG with Iodine-123 (¹²³I) allows for scintigraphic assessment of sympathetic activity.⁴¹ Because increased release of NE (and subsequently decreased mIBG uptake) is one of the first neurohumoral responses before cardiac output declines (Figure 1.2), ¹²³I-mIBG scintigraphy is thought to detect subclinical AIC in an early phase.^{43, 44}

In general, the sympathetic neuronal integrity is semi-quantitatively assessed by the heart-to-mediastinum (H/M) ratio and the washout (WO) on planar ¹²³I-mIBG scintigraphy. Normal myocardium will portray a high H/M ratio (i.e. the uptake of ¹²³I-mIBG in the heart is high) and a low WO (i.e. once taken up, a substantial amount of ¹²³I-mIBG stays in the nerve endings). In case of increased sympathetic activity, the H/M ratio will be low and the WO high (Figure 1.3).^{41, 43} Normal values of H/M ratios and WO vary widely in literature, because of differences in camera details, acquisition protocols and quantification methods.⁴⁵ Standardization of these parameters has been proposed to minimize these variations.⁴⁶ Detailed information on the acquisition parameters are provided in the relevant chapters.

A decreased ¹²³I-mIBG H/M ratio is a strong prognostic marker and is an independent predictor of ventricular tachyarrhythmia, sudden cardiac death and outcome of ICD-therapy.⁴⁷⁻⁵¹ Furthermore, an increased WO has been associated with an adverse prognosis.^{51, 52}

Figure 1.1 Schematic display of norepinephrine (NE) and ^{123}I -mIBG (*m*) sympathetic pathway.

A. In response to a stimulus, NE-containing vesicles are released into the synaptic cleft. There, NE binds to mainly β_1 -receptors on the postsynaptic surface, which enhance adenylyl cyclase (AC) activity through G protein (G) activation. NE is recycled by the uptake 1 pathway for storage or degradation by mono amine oxidase (MOA).

B. Guanethidine, an inactive neurotransmitter that resembles NE, is chemically modified and labeled with ^{123}I , becoming ^{123}I -mIBG. When this radioactive compound is available in the circulation, it is taken up and stored in the same way as NE, but cannot be catabolised by MOA. Therefore, ^{123}I -mIBG is retained in sufficient concentrations to allow imaging with a gamma camera.

Although it has been shown that ^{123}I -mIBG uptake decreases after use of anthracyclines and this appears before morphologic changes and alterations in LVEF, clinical studies on this subject are lacking.^{6,38}

(2D strain) echocardiography

Echocardiography has been the main cardiac imaging technique for a long time, because it is widely available, noninvasive, fast, cheap and relatively easy to perform. Both systolic and diastolic function can be assessed. However, it is sometimes difficult to interpret the images and only considerable LVEF deterioration can be detected.³⁹ Furthermore, the reproducibility of 2D echocardiographic parameters is only moderate.⁵³

2D strain myocardial strain imaging is an adapted form of echocardiography and measures the relative deformation of cardiac tissue in the longitudinal, radial and circumferential axis (Figure 1.4).⁵⁴ Since strain can differentiate active from passive movement, subtle regional

Figure 1.2 Flow chart AIC and decreased ^{123}I -mIBG uptake.

Flow chart of the cascade that leads to a decreased storage of ^{123}I -mIBG in the sympathetic nerve endings.

differences can potentially be detected.⁵⁵ 2D strain has already proven its value in the monitoring of patients treated for breast cancer, detecting cardiotoxicity with higher sensitivity than conventional echocardiography.⁵⁶⁻⁵⁸ Strain rate, the temporal derivative of strain, has been evaluated in several studies, but results vary and the usefulness of strain rate is therefore still under debate.^{56,57,59}

Biomarkers

When the heart suffers damage, whether it is due to ischemia or medication, several compensatory mechanisms will commence. A comprehensive overview of these reactions is given by Braunwald, whose cytokine hypothesis is depicted in Figure 1.5.⁶⁰ The release of a range of different cytokines opens up new possibilities to detect a heart in distress. Many of these cytokines can be measured and therefore are entitled as biomarkers.

Figure 1.3 Examples of H/M ratios.

- A.** Normal ^{123}I -mIBG study: High H/M ratio of approximately 3.3.
B. Decreased ^{123}I -mIBG uptake in damaged myocardium: Low H/M ratio of approximately 1.6.

The use of biomarkers in cardiac screening has been widespread in recent years. The myofibrillar protein troponin I (TnI), for example, is used to detect acute myocardial ischemia and N-terminal pro brain natriuretic peptide (NT-proBNP), an inactive precursor of the active BNP, is measured to diagnose and monitor cardiac failure. Furthermore, C-reactive protein (CRP) has shown to identify asymptomatic subjects who were at high risk for the future development of heart failure.^{39, 60}

To identify the most promising biomarkers, Morrow et al. have formulated three general criteria that a biomarker should possess:

- 1) Accurate, repeated measurements must be available at reasonable cost and with short turnaround times.
- 2) It must provide information that is not already available by careful clinical assessment.
- 3) It must aid in clinical decision making.⁶¹

Figure 1.4 Strain axes.

Schematic of strain (rate) imaging axes in the long left ventricular axis. Strain measures the relative deformation of the LV wall in the longitudinal (L), radial or transversal (R) and circumferential (C) axis. Figure adapted and reproduced with permission of Støylen.

Besides these general attributes, minor, disease-specific criteria can be added. In the case of AIC, additional criteria are a possible detection early in the cardiotoxic process and a high specificity. CRP, for example, does not classify as a promising biomarker, since it has shown to be elevated in dozens of other diseases, thus lacking specificity.⁶⁰ Research on NT-proBNP and TnI is still ongoing and other biomarkers are suggested regularly. The current challenge is to identify the biomarker that best suits our needs.

MUGA scintigraphy

MUGA scintigraphy is a well-established method to determine the left ventricle (LV) function through the injection of a radiolabelled blood pool agent like human serum albumin. Alternatively, one can label patients' own erythrocytes to image the circulation.⁶² The MUGA technique has been used for over four decades and its main advantage is the high reproducibility and simplicity to interpret.⁵³

Figure 1.5 The cytokine hypothesis.

According to the cytokine hypothesis of heart failure, proinflammatory cytokines (tumor necrosis factor α , interleukin-1, interleukin-6, and interleukin-18) are produced by the damaged myocardium; this production is enhanced by stimulation of the sympathetic nervous system. Injured myocardium, as well as skeletal muscle that is hypoperfused because of reduced cardiac output, activates monocytes to produce the same cytokines, which act on and further impair myocardial function (dashed lines). Cytokines from these several sources are also released into the bloodstream. The stressed myocardium releases natriuretic peptides, denoted in red; their release improves the circulation. Figure reproduced with permission of Braunwald. © Massachusetts Medical Society.

In the vast majority of MUGA scans, clinicians are only interested in monitoring the systolic function of the heart. However, in AIC it is known that DD occurs before systolic dysfunction (SD) due to differences in the susceptibility of endocardial and mid-myocardial tissue for anthracycline damage.⁶³⁻⁶⁵ Peak filling rate (PFR) and time to peak filling rate (t-PFR) are diastolic parameters that can be derived from the time-volume curve, which is computed for every MUGA scan (Figure 1.6) and also has excellent reproducibility.^{53,62} For TIC, it is not yet known whether SD or DD occurs first.

Figure 1.6 LV time-volume curve.

For every MUGA scan an average time (t) - volume (V) curve is computed, which depicts the physiology of an average cardiac cycle. The curve can be divided in a systolic part (starting at end-diastole, ED; ending at end-systole, ES), in which the left ventricle volume decreases through ventricle contraction and a diastolic part (starting at end-systole and ending at end-diastole), in which the left ventricle volume increases through ventricle relaxation. The maximum speed the LV empties is considered the peak ejection rate (PER) and the time that is needed to achieve PER is called the time to PER (t-PER). Similarly, the maximum speed the LV is filled is considered the peak filling rate (PFR) and the time that is needed to achieve PFR is called the time to PFR (t-PFR). A combination of different values for different parameters characterizes cardiac physiology.

Cardiotoxicity in childhood cancer survivors

A group of patients that deserves special attention with regards to cardiotoxicity are childhood cancer survivors. In 2010, the five year survival rate for these patients was 83%. Of these patients, a majority has been treated with anthracyclines, often in combination with radiotherapy, and is therefore at high risk of long term AIC/RIC. By the age of 45, the cumulative incidence for heart failure in these patients is 4.5%, compared to 0.3% for their siblings.³¹ Although many preventive strategies have been proposed, including the use of dexrazoxane and carvedilol, no effective regimen has been determined and the screening for patients at highest risk still is imperative.³¹

As for adult cancer survivors, different methods for the early detection of AIC in children have been studied. Lipshultz et al. evaluated children with acute lymphoblastic leukaemia treated with doxorubicin for levels of troponin T, NT-proBNP and high-sensitive CRP and showed a correlation with echocardiographic parameters indicating cardiotoxicity.⁶⁶ Mavinkurve-Groothuis showed that strain (rate) parameters differed significantly between asymptomatic childhood cancer survivors and healthy controls and also correlated with several conventional echocardiographic measurements.⁶⁷ Furthermore, assessment of LV function by cardiac magnetic resonance imaging (CMR) is increasingly used in paediatric patients.⁶⁶

¹²³I-*m*IBG scintigraphy is already used in paediatric patients that suffer from neuroblastoma.⁶⁸ Despite that, the (semi-quantitative) use of cardiac ¹²³I-*m*IBG has only been studied in very small or specific patient groups.⁶⁹⁻⁷¹ However, a Japanese study in 33 patients with various cardiac diseases showed encouraging results on the role of the H/M ratio and WO.⁷² Unfortunately, till date these results have not triggered additional research on this subject.

Experimental imaging of cardiac mechanisms

Since the optimal method for the early detection of AIC has not yet been established, studies concentrate on a broad spectrum of different radiopharmaceuticals that could be of value, both in a clinical and an experimental fashion. One of the main objectives of experimental studies, next to discovering new imaging biomarkers, is further elucidating the pathophysiology of the cardiotoxic process and the different (compensatory) mechanisms that are involved, in a matter analogous to Braunwald.⁶⁰

Apoptosis is one of the main pathological responses of (cardiac) cells to damage by anthracyclines.^{10, 38} Imaging of apoptosis would therefore be a logical approach to depict early cell damage before this comes clinically apparent. Annexin V, labelled with Technetium-99-*metastable* (^{99m}Tc), is a radiopharmaceutical that can be used to assess apoptotic cell death, since Annexin V binds to certain molecules that are increasingly expressed on the outer membrane of apoptotic cells.⁷³ The process of cell membrane disintegration, which occurs in myocardial infarction and necrosis, but not in apoptosis, can be imaged by ^{99m}Tc-glucaric acid, since this small molecule enters the cell and binds to nuclear histones.⁷⁴ Furthermore, glucose metabolism of the heart, depicted by the well-known oncologic tracer Fluorine-18-fluorodeoxyglucose (¹⁸F-FDG), differs in patients treated with anthracyclines, probably as a compensatory mechanism for cardiac damage.⁷⁵ Above that, mitochondrial membrane disruption induced by anthracyclines might be a potential target for imaging with ^{99m}Tc-sestamibi.⁷⁶

The abovementioned mechanisms are all results of different small studies. The interrelationship of these mechanisms and their place in the general cardiotoxic process has not been determined. However, establishing a general hypothesis on the mechanisms of cardiotoxic cell damage is a key feature in optimizing prevention and treatment of AIC.

Outline of the thesis

As the survival of breast cancer patients is improving, and will presumably improve further with the development of patient-tailored treatment, the early detection and management of adverse events that occur due to therapeutic interventions becomes more important. Especially treatments with potential side effects that can be as damaging as induced by anthracyclines and, to a lesser extent trastuzumab, have to be monitored carefully. In the current thesis different promising methods to monitor patients under treatment are studied to detect signs of cardiac deterioration as early as possible, both in a clinical and an experimental setting. Furthermore, the pitfalls of cardiac ¹²³I-*m*IBG scanning in children are addressed.

Chapters 2, 3 and 4 address the findings of the TOXTAC study, in which a cohort of breast cancer survivors treated with the chemotherapeutic regimen including Taxanes, Adriamycin and Cyclophosphamide (TAC) was assessed with different methods for early AIC detection one year after treatment. **Chapter 2** describes the use of echocardiographic strain imaging in the early detection of AIC, while in **Chapter 3** several promising biomarkers are evaluated. In **Chapter 4** multiple ¹²³I-*m*IBG scintigraphic parameters are compared for intermethod and interobserver variability. The most robust parameter was then correlated with strain imaging, biomarkers and conventional echocardiography. In **Chapter 5** diastolic and systolic dysfunction during trastuzumab therapy are retrospectively studied through multigated radionuclide angiography in a cohort of breast cancer patients.

Chapter 6 and 7 concentrate on ¹²³I-*m*IBG imaging in paediatric patients. In **Chapter 6**, the influence of catecholamines on ¹²³I-*m*IBG parameters in patients with neuroblastoma is described. **Chapter 7** addresses the use of different regions of interest for the calculation of these scintigraphic parameters.

In **Chapter 8**, an experimental animal study on promising radiopharmaceuticals will be presented, which are correlated with histological results on cardiac mice tissue.

Chapter 9 comprises the general discussion and conclusions. Furthermore, directions for future research on the early detection of cancer treatment induced cardiotoxicity are proposed.

Chapter 10 consists of the English and Dutch summary of this thesis.

2

Early myocardial deformation abnormalities in breast cancer survivors

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Abstract

Purpose: To evaluate the role of 2D myocardial strain (rate) imaging in the detection of early subclinical cardiotoxicity in breast cancer survivors treated with an anthracycline-based chemotherapeutic regimen.

Methods: 57 adult breast cancer survivors were analysed one year after therapy. All patients underwent biomarker analysis and 2D echocardiography consisting of conventional echocardiographic and strain (rate) parameters.

Results: Conventional echocardiographic values were normal. Global longitudinal strain (GLS) was normal, but 18% of patients showed a >2 SD decrease when individually compared to reference values. This subgroup showed a decrease in end systolic and end diastolic volumes and an increase in left ventricular mass. Radial and circumferential strain rates were significantly decreased in the whole study group.

Conclusion: 2D myocardial strain (rate) imaging showed abnormalities in breast cancer survivors, while conventional echocardiographic values remained normal, rendering 2D myocardial strain (rate) imaging an interesting tool for the early detection of anthracycline-induced cardiotoxicity.

Introduction

More than four decades after their discovery, anthracyclines remain among the most widely prescribed anticancer agents.⁵⁶ It is well known that anthracycline treatment can be compromised by insidious cardiomyopathy and heart failure. Incidences of subclinical heart failure are reported to be even up to 65% in asymptomatic survivors of childhood cancer.⁷⁷⁻⁸⁰ A study in elderly breast cancer survivors treated with anthracyclines showed a hazard ratio of 1.26 for the development of congestive heart failure.⁷⁹ The mechanism by which this takes place is not yet elucidated and it is therefore hard to prevent its occurrence.^{40, 81}

Anthracycline-induced cardiotoxicity (AIC) can be divided into acute (during or immediately after treatment), early-onset (<1 year) and late-onset (>1 year).^{4, 77} Acute cardiotoxicity is often reversible, while chronic cardiotoxicity is not. However, it is known that acute cardiotoxicity is a risk factor for the occurrence of late-onset cardiotoxicity.^{4, 82} Early detection of chronic AIC is therefore clinically relevant.

While treatment for breast cancer has improved and the number of survivors increases every year, an adequate tool to detect subjects at risk for late AIC is still lacking.⁴⁰ When adult patients are also treated with other cardiotoxic agents like taxanes or trastuzumab, early detection of cardiotoxicity might be even more important.⁸¹ Currently, resting left ventricular ejection fraction (LVEF) by two-dimensional (2D) echocardiography or multigated radionuclide angiography (MUGA) is the key parameter used to identify and monitor AIC in adults.^{38, 39, 56} However, both methods have numerous technical limitations and only measure global function, which is a late sign of myocardial damage.^{55, 56, 83} Several recent studies have shown that myocardial strain imaging may provide a more sensitive approach to detect early alterations of left ventricle systolic function.^{56, 57, 59, 67, 84, 85}

The aim of the current study is to evaluate the role of 2D myocardial strain imaging in detecting early subclinical cardiotoxicity (as compared to conventional 2D echocardiography) in breast cancer patients who were treated with a combined chemotherapeutic regimen including docetaxel (Taxotere®), doxorubicin (Adriamycin®) and cyclophosphamide (Endoxan®), abbreviated as TAC.

Methods

Study population

Between October 2010 and May 2012 all female adults with breast cancer who completed (neo-)adjuvant treatment with TAC longer than one year ago, were identified from the database of the Dutch Comprehensive Cancer Centre (IKO) and were invited to take part

in the present study. Exclusion criteria were: heart disease at diagnosis (i.e. heart failure, ischemic heart disease); evidence of breast cancer recurrence or metastasis and renal failure at the time of cardiac evaluation.

A detailed medical history and physical examination was obtained from all patients, with special attention to risk factors and signs and symptoms of cardiac disease. Current medication use was noted. A standard 12-lead electrocardiogram was performed and analysed for signs of cardiac disease and rhythm disturbances. The study was approved by the local medical ethics committee and informed consent was obtained from all patients.

Echocardiography

All patients underwent a transthoracic 2D echocardiogram in supine and lateral position at rest. The echocardiogram was performed by an experienced heart failure cardiologist (LB). Images were obtained with a 5.0-MHz transducer, using the Vivid 7 echographic scanner (GE, Vingmed Ultrasound, Horten, Norway). Quantification of cardiac chamber size, ventricular mass and systolic and diastolic left ventricular function were performed in accordance with the recommendations for chamber quantification by the American Society of Echocardiography's Guidelines and Standard Committee and the Chamber Quantification Writing Group.^{86, 87}

An M-mode echocardiogram was performed in the parasternal long and short axis views to measure the internal dimensions of the left ventricle at end-diastole (LVIDd) and end-systole (LVIDs), the posterior and septal wall thickness at end-diastole (LVPWd, IVSd) and the left ventricular mass (LVM). The latter was calculated by the formula of Devereux.⁸⁸ Two-dimensional apical two- and four-chamber gray scale images were made to measure left ventricular volume at end diastole (LVEDV) and end systole (LVESV) and left atrial end diastolic volume (LAEDV). Measurements of LVEDV, LVESV, LVM and LAEDV were indexed by body surface area (BSA).

Left ventricle systolic function was measured using the modified Simpson's ejection fraction (EF). Left ventricular diastolic function was evaluated using LAEDV, early (E) and late (A) diastolic transmitral peak flow velocity (E/A ratio), the systolic to diastolic pulmonary vein peak flow velocity (PV S/D-ratio) and early diastolic transmitral peak flow velocity (E) to early diastolic annular velocity (e') ratio (E/e' ratio). The E/A ratio and PV S/D ratio were obtained by pulse Doppler at the mitral valve inflow and the pulmonary vein inflow respectively. The early diastolic annular velocity was measured at the basal segment of the lateral left ventricular wall.⁸⁹ Abnormal diastolic function was defined as abnormal LAEDV/BSA or E/e' ratio.

Strain analysis was done using 2D gray scale images taken in the parasternal apical four-chamber, apical two-chamber, mid-cavity short-axis (at the level of the papillary muscle) and basal short-axis views. A sector scan angle of thirty to sixty degrees was chosen and frame rates of 70 Hz or more were used.⁹⁰ Cine loops of three cardiac cycles triggered by the R-wave of the QRS-complex were digitally saved. Offline analysis was performed using software for echocardiographic quantification (EchoPAC 6.1.0, GE Medical Systems, Horten, Norway). Timing of aortic valve closure (AVC) and mitral valve opening (MVO) was used to indicate end-systole and start of diastole respectively.

Myocardial segments were named and localized according to the statement of the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association.⁹¹ Manual tracking of the endomyocardial borders was performed at the end-systolic frame. An automatic generation of the second epicardial tracing was created by the software, which also automatically divided the image into six equal segments. Quality of the tracking was verified for each segment and adjusted when needed. The three consecutive cardiac cycles were analysed for each segment. Figure 2.1 shows a composite figure with the region of interest (left side) and graphic depiction of longitudinal strain in four-chamber long axis view.

Strain values are dimensionless and are expressed in percentage. Strain rate is the temporal derivative of strain and is expressed as 1/s. The average values of peak systolic longitudinal, radial and circumferential strain and strain rate of the three imported curves were calculated without correction for the length of the cardiac cycle since it is known that the systolic phase is relatively constant if heart rate changes less than ten percent.⁹² Cardiac cycles with a length more than ten percent different from the mean length of the three cardiac cycles were excluded for further analysis. Global longitudinal myocardial strain (GLS) and strain rate (GLSR) were calculated by averaging the six segments of the four-chamber long-axis view. Global radial and circumferential myocardial strain (GRS, GCS) and strain rate (GRSR, GCSR) were calculated by averaging the six segments of the mid-cavity short-axis view.

To evaluate the conventional echocardiographic values obtained in our patient group, we used the widely implemented reference values as described by the American Society of Echocardiography.^{86, 87} The obtained 2D strain imaging values were stratified as normal or abnormal according to the HUNT-study by Dalen et al.⁹³ Values were considered abnormal when deviating >2 SD from mean.

Biochemical analysis

Of each patient venous blood samples were obtained at the same time of echocardiographic study. Routine blood chemistry was performed in all patients. Ethylenediamine-tetraacetic acid (EDTA) blood was stored on ice for determination of anemia, kidney

Figure 2.1 Composite figure with the region of interest (left side) and graphic depiction of longitudinal strain in four-chamber long axis view.

function, HbA1c, troponin I (TnI) and NT-proBNP. The latter was determined using the immulite 2500 chemiluminescence immunoassay system (Siemens Medical Solution Diagnostics, Deerfield, IL, USA). Age- and gender-specific reference values for NT-proBNP were used as established by Hess et al.⁹⁴ Other normal values were derived from local laboratory references.

Statistical analysis

Characteristics of the study sample such as age at study, BSA and cumulative anthracycline dosage were summarized by using median and range. Conventional echocardiographic and strain (rate) parameters were expressed by using mean and standard deviation. Differences between patients and reference values from literature were studied using the two sample t-test.

Statistical analyses were performed using the SPSS Statistics for Windows, version 20.0. A *p*-value <0.05 was considered to indicate significance.

Results

Patient characteristics

58 patients met the inclusion criteria and were included in the study. One patient was excluded because of severe aortic stenosis. In the remaining 57 patients the median time of evaluation was 12.6 (range 12-14) months after TAC treatment. None of the patients received dexrazoxane as cardioprotective agent, nor was using trastuzumab before or at the time of inclusion. Furthermore, none of the patients had a history of acute cardiotoxicity immediately following anthracycline treatment. The characteristics of the study sample are shown in Table 2.1.

All patients were in New York Heart Association (NYHA) class I and did not show clinical signs and symptoms of heart failure. One patient had an already known left bundle branch block on ECG.

Biochemical analysis

Biochemical analysis showed elevated HbA1C in eight patients (14%), mild anemia in one patient (hemoglobin 7.3 mmol/L) and normal kidney function and TnI levels in all patients. Abnormal NT-proBNP levels were found in almost 16% of the patients (median value 119, range 26-818 pg/ml). Of these patients only one had an abnormal GLS.

Conventional echocardiographic parameters

Conventional echocardiographic data of the whole sample is presented in Table 2.2. Two patients had a slightly abnormal LVEF (one 54% and one 53%), with a mean of 62%±7. Mean PV S/D ratio was within normal values (1.4±0.4, compared to a reference value of 1.2±0.2, *p* <0.01). Other conventional echocardiographic values were also not different from reference values.

Myocardial 2D strain parameters

2D myocardial strain (rate) measurements of the whole study sample are presented in Table 2.2. Longitudinal strain (rate) data was obtained in almost all patients (N = 55, 96%). GRS and GCS were acquired in 41 patients (72%) and GRSR and GCSR in forty patients (70%), due to insufficient image quality in the remainder of patients.

The mean GLS of the whole study group (-17.8±2.8%) was not different from the 40-60 years control group from Dalen's HUNT study (-17.6±2.1%).⁹³ However, the mean GLSR (-0.88 ±0.16 s⁻¹) was significantly decreased in comparison with the HUNT group (-1.06±0.13 s⁻¹, *p* <0.05). Interestingly, ten patients (18%) had an abnormal GLS defined as >2 SD of the reference values described by Dalen et al.⁹³ Of these patients, none had an abnormal LVEF (Table 2.3). The mean LVEDV/BSA of this subgroup was 36.8±8.2 ml/m², while patients with a normal GLS showed a mean LVEDV/BSA of 47.3±8.2 ml/m² (*p* <0.01). The mean values of

Table 2.1 Patient characteristics of the study group (N = 57).

Median age at study in years (range)	51 (36-69)
Median time after treatment in months (range)	12.6 (12-14)
Mean BSA in m ² (SD)	1.89 (0.19)
Tumor side (N)	
Left (%)	27 (47)
Right (%)	23 (40)
Both (%)	7 (12)
Radiation location (N)	
Left or both (%)	21 (37)
Right (%)	16 (26)
Mean total radiation left thorax in Gy (SD)	58 (10.6)
Median cumulative anthracycline dose in mg/m ² (range)	300 (250-300)
Risk factors (N)	
Smoking (%)	12 (21)
Hypertension (%)	16 (28)
Family history (%)	26 (46)
Hypercholesterolemia (%)	4 (7)
HbA1c > 6 mmol/L (%)	8 (14)

BSA = body surface area, SD = standard deviation.

LVESV/BSA were 14.8±3.8 ml/m² for the abnormal GLS group and 18.1±4.4 ml/m² for normal GLS group, respectively ($p < 0.05$). Furthermore, mean LVM/BSA was 73.5±15.3 g/m² in the normal strain group, compared to 84.8±18.8 g/m² in the abnormal strain group ($p < 0.05$). Other conventional echocardiography values did not significantly differ (Table 2.3).

Mean GRSR in the whole study sample was 1.5±0.4 s⁻¹, mean GCSR -1.0±0.2 s⁻¹. When compared to normal values for GRSR and GCSR (2.2±0.56 s⁻¹ and -1.72±0.3 s⁻¹ respectively), as delineated by Stoodley et al.,⁵⁶ both showed a statistically significant difference ($p < 0.01$). Mean GRS and GCS were not significantly different (Table 2.2).

Table 2.2 Conventional and strain (rate) echocardiographic values.

	Study population		Reference value	
	N	Mean (SD)		
Conventional values				
EF (%)	57	62.8 (6.2)	≥ 55	
LVEDV/BSA(ml/m²)	56	45.8 (9.2)	35-75	
LVESV/BSA in (ml/m²)		17.5 (4.7)	12-30	
LVIDd (cm)	57	4.7 (0.5)	3.9-5.3	
IVSd (cm)		0.89 (0.12)	0.6-0.9	
LVPWd (cm)		0.89 (0.12)	0.6-0.9	
LVM/BSA (g/m²)		75.8 (16.2)	44-88	
LAEDV/BSA (ml/m²)	55	21.9 (5.7)	22 (±6)	
E/e' ratio	51	6.4 (2.3)	< 8	
E/A ratio	56	1.2 (0.3)	1.3 (±0.3)	
Pulmonary vein S/D ratio	52	1.4 (0.4)	1.2 (±0.2)*	
2D strain (rate) values				
	N	Mean (SD)	Mean (SD)	
			Dalen et al.	Stoodley et al.
GLS (%)	55	-17.8 (2.8)	-17.6 (2.1)	-17.8 (2.1)
GLSR (1/s)		-0.88 (0.16)	1.06 (0.13)†	-0.87 (0.14)
GRS (%)	41	38.6 (9.4)		40.5 (11.4)
GRSR (1/s)	40	1.5 (0.4)		2.20 (0.56)*
GCS (%)	41	-19.1 (4.3)		-20.3 (2.6)
GCSR (1/s)	40	-1.0 (0.2)		-1.72 (0.3)*

EF = ejection fraction, LVEDV = left ventricular end-diastolic volume, BSA = body surface area, LVESV = left ventricular end-systolic volume, LVIDd = left ventricle internal dimension at diastole, IVSd = interventricular septum at diastole, LVPWd = left ventricular posterior wall at diastole, LVM = left ventricular mass, LAEDV = left atrial end-diastolic volume, E/e' ratio = early diastolic transmitral peak flow velocity (E) to early diastolic annular velocity (e') ratio, E/A ratio = early (E) and late (A) diastolic transmitral peak flow velocity ratio, S/D ratio = systolic to diastolic ratio, GLS = global longitudinal strain, GLSR = global longitudinal strain rate, GRS = global radial strain, GRSR = global radial strain rate, GCS = global circumferential strain, GCSR = global circumferential strain rate.

Conventional echocardiographic reference values by Lang et al. and Nagueh et al.^{86, 89} Strain (rate) reference values by Dalen et al. and Stoodley et al.^{56, 93}

* $p < 0.01$. † $p < 0.05$

Table 2.3 Subgroup analysis of conventional and strain (rate) values in patients with abnormal GLS.

	Normal strain		Abnormal strain	
	N	Mean (SD)	N	Mean (SD)
Conventional values				
EF (%)	45	62.4 (5.6)	10	62.4 (5.8)
LVEDV/BSA(ml/m ²)		47.3 (8.2)	9	36.8 (8.2)*
LVESV/BSA in (ml/m ²)		18.1 (4.4)		14.8 (3.8)†
LVIDd (cm)		4.6 (0.5)	10	4.9 (0.5)
IVSd (cm)		0.89 (0.11)		0.88 (0.17)
LVPWd (cm)		0.87 (0.11)		0.95 (0.15)
LVM/BSA (g/m ²)		73.5 (15.3)		84.8 (18.8)†
LAEDV/BSA (ml/m ²)	43	21.7 (5.7)		23.2 (6.2)
E/e' ratio	40	6.4 (1.6)		6.8 (4.2)
E/A ratio	44	1.21 (0.36)		1.07 (0.19)
Pulmonary vein S/D ratio	42	1.38 (0.38)	8	1.30 (0.22)
2D strain (rate) values				
GRS (%)	33	39.0 (9.2)	7	37.0 (11.6)
GRSR (1/s)	32	1.47 (0.36)		1.40 (0.36)
GCS (%)	33	-19.3 (4.3)		-18.6 (4.9)
GCSR (1/s)	32	-1.04 (0.23)		-1.07 (0.19)

EF = ejection fraction, LVEDV = left ventricular end-diastolic volume, BSA = body surface area, LVESV = left ventricular end-systolic volume, LVIDd = left ventricle internal dimension at diastole, IVSd = interventricular septum at diastole, LVPWd = left ventricular posterior wall at diastole, LVM = left ventricular mass, LAEDV = left atrial end-diastolic volume, E/e' ratio = early diastolic transmitral peak flow velocity (E) to early diastolic annular velocity (e') ratio, E/A ratio = early (E) and late (A) diastolic transmitral peak flow velocity ratio, S/D ratio = systolic to diastolic ratio, GLS = global longitudinal strain, GLSR = global longitudinal strain rate, GRS = global radial strain, GRSR = global radial strain rate, GCS = global circumferential strain, GCSR = global circumferential strain rate.
Conventional echocardiographic reference values by Lang et al. and Nagueh et al.^{86, 89} Strain (rate) reference values by Dalen et al. and Stoodley et al.^{56, 93}
* $p < 0.01$. † $p < 0.05$.

In 22 patients (38.6%) an abnormal NT pro-BNP, a 2D echocardiographic diastolic dysfunction or an abnormal longitudinal strain (rate) was detected. Four patients (7.0%) showed abnormal values for two of these variables: two patients had an abnormal NT pro-BNP and strain and two a diastolic dysfunction and an abnormal strain. In only one

patient (1.8%) an abnormal value for all three variables was measured. The remaining patients (N = 30, 52.6%) demonstrated no abnormal variables.

Discussion

In this study we evaluated the role of myocardial strain (rate) imaging in the detection of subclinical AIC one year after treatment with TAC chemotherapy. The main finding of this study is that 18% of patients showed a decreased GLS and significant decrease in global strain rates, while echocardiographic LVEF and other conventional parameters remained normal. This suggests that strain (rate) measurements might be more sensitive in determining left ventricular dysfunction than conventional values, such as LVEF.

Several studies have already underlined the sensitivity of myocardial strain to detect AIC.^{56-59, 84, 95, 96} Most studies highlight the role of GLS in particular,^{58, 84, 95} while GRS and GCS are thought to be less robust and reproducible.^{56, 97} However, some evidence suggests that GRS is capable of detecting AIC.^{56, 96} Results on strain rate measurements are equivocal. Jurcut et al. observed a significant decrease in GLSR and GRSR in a small group of patients.⁵⁹ Recent work by Florescu et al. shows a decrease of GLSR after epirubicin, but a constant GRSR and GCSR.⁵⁷ Stoodley et al. do not observe a decrease in any of the strain rate measurements.⁵⁶

In our study, global strain measurements of the whole group did not significantly differ from reference values. There are several explanations for these discrepant results related to other studies. First, some studies include patients that receive or have received trastuzumab.^{58, 84, 95} Trastuzumab is known to affect cardiac function negatively and can therefore increase cardiotoxicity and bias the predictive value for AIC.⁹⁸

Furthermore, the moment of strain (rate) evaluation in our study is relatively late compared to other studies. For example, Sawaya et al. evaluated their patients after median 3.5 months, while Stoodley et al. and Jurcut et al. evaluated their patient group during or directly after chemotherapy.^{56, 58, 59} Recent studies by Sawaya et al. and Hare et al. described global longitudinal and radial strain (rate) at different time points after anthracycline chemotherapy and during trastuzumab treatment.^{84, 99} Both showed an initial drop of GLS followed by stabilisation. Hare et al. found a mild but progressive decrease of GRS, Sawaya et al. a rather large progressive decrease, recovering after nine to twelve months.^{84, 99} After an initial mild drop, GCS normalised after six to nine months.⁸⁴ GLSR and GRSR showed a marked and persistent decrease.⁹⁹ This could imply that strain (rate) measurements differ in the period after chemotherapy and that GLS and GLSR tend to decrease directly after chemotherapy, but normalise in time.

While global strain measurements were not significantly abnormal compared to reference values, global strain rate measurements were. This could imply that strain rate is more sensitive in detecting early-onset cardiotoxicity or that strain rate is still reduced one year after chemotherapy, while strain is not. The normal E/A ratio of our patients suggested that load-dependency was not the reason for the difference in strain rate parameters between our sample and the reference population, nor was it between the subgroups with our without strain abnormalities.

An intriguing finding in our study is that patients with an abnormal longitudinal strain had a significantly decreased LVEDV/BSA and LVES/BSA, while LVM/BSA was significantly increased. This decrease in cardiac volumes and increase in ventricular mass is discordant with the generally accepted theory of late-onset AIC, which suggests loss of myocytes and a dilated cardiomyopathy.^{39, 100} Possible explanations for this are an early hypertrophic remodeling phase in the process of cardiomyopathy or the induction of a restrictive cardiomyopathy by anthracyclines. Restrictive cardiomyopathy after treatment with anthracyclines has been described in a group of 115 doxorubicin-treated long term acute lymphatic leukemia survivors in which a progressive decline of the left ventricular internal dimension was seen.¹⁰¹ This study also described an initial increase of LVM in the first years after doxorubicin in the high-dose (>400 mg/m²) subgroup. Two pathology-based studies described both hypertrophy and fibrosis in survivors of childhood cancer suffering from late-onset cardiotoxicity, which suggests a restrictive component of cardiomyopathy.^{102, 103}

In our patient group, the increased LVM might be aggravated by concomitant post-radiation effects. Breast or chest wall radiotherapy is a known risk factor for cardiotoxicity.^{32, 104} This is partly due to fibrosis of endomyocardial tissue, which leads to a restrictive cardiomyopathy. However, thanks to dose-decreasing approaches, radiation-induced cardiotoxicity nowadays comprises a relative risk of 1.2 to 3.5.¹⁰⁵ Nevertheless, this is still a considerable risk, and combination regimens might increase or speed up the process of cardiotoxicity.

Furthermore, TAC chemotherapy also includes docetaxel and cyclophosphamide. Docetaxel is thought to potentiate the cardiotoxic side effects of doxorubicin,¹⁰⁶ but evidence is scarce. The responsible mechanism is not known and cardiotoxicity is less than associated with paclitaxel.^{107, 108} Cyclophosphamide is known for its acute cardiotoxicity in high dose^{109, 110} but this is not expected to influence cardiac function in the late-onset setting one year post treatment.

In the current study 16% of patients had an abnormal NT-proBNP compared to (age and gender adjusted) reference values, while TnI levels were normal in all patients. A recent study of Mavinkurve et al. shows identical percentages in the long term follow-up of

childhood cancer survivors treated with anthracyclines.⁶⁷ Several studies on this subject in adults show a broad array of values, ranging from 2 to 62%.^{111, 112}

In the current study we compared to control groups of Stoodley et al. and Dalen et al.^{56, 93} Stoodley's group comprised 52 participants, which were all breast cancer patients before treatment with anthracyclines. The HUNT study of Dalen et al. registered global and regional longitudinal strain (rate) reference values of 673 randomly selected healthy women, of which 336 in the age group forty to sixty years. Both groups had fairly similar patient characteristics and the method for obtaining the strain (rate) measurements was comparable.

2D echocardiographic myocardial strain imaging is widely available, cheap, easy to perform, noninvasive and minimizes patients' discomfort. We used the method to evaluate a relatively large and very homogenous patient group, treated with a similar type of chemotherapy and in absence of trastuzumab. The study was done in a narrow time interval after chemotherapy, leading to results that can be generalized. We observed decreased strain (rate) with preservation of conventional values one year after therapy. Myocardial strain imaging might therefore become a promising technique in the evaluation of AIC. A prospective follow-up of the patients for a longer time interval is needed in order to conclude whether 2D strain (rate) is indeed a more sensitive tool. We believe that strain (rate) imaging can be an interesting parameter to investigate together with the assessment of the conventional echocardiographic parameters.

Limitations

Due to the lack of a baseline echocardiographic measurement (e.g. pre- or post-anthracyclines), we could not assess cardiac function over time. However, the aim of the study was not to detect the quantity of change in strain (rate), but to observe a strain (rate) decrease with preservation of conventional values.

Because of the limited amount of patients included in the study, risk factors in the subgroup analysis could not be analysed.

Conclusion

One year after TAC chemotherapy, 2D myocardial strain (rate) imaging showed myocardial deformation abnormalities in breast cancer survivors, while the EF remained normal.

3

New biomarkers for early detection of cardiotoxicity after treatment with docetaxel, doxorubicin and cyclophosphamide

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Abstract

Purpose: Assessing a diverse biomarker panel (NT-proBNP, TNF- α , galectin-3, IL-6, Troponin I, ST2 and sFlt-1) to detect subclinical cardiotoxicity after treatment with anthracyclines.

Methods: Of 55 breast cancer patients biomarkers were assessed and echocardiography was performed one year after treatment with anthracyclines.

Results: 29.1% of patients showed abnormal biomarker levels: NT-proBNP in 18.2%, TNF- α and Galectin-3 in 7.3%. IL-6, troponin I, ST2 and sFlt-1 were normal in all patients. A correlation between left ventricular ejection fraction (LVEF) and NT-proBNP was observed ($r = -0.564$, $p < 0.01$).

Conclusion: The evaluated biomarkers do not contribute to early detection. Future research should focus on NT-proBNP.

Introduction

Currently, chemotherapy with docetaxel, doxorubicin and cyclophosphamide (TAC) is one of the commonly used treatment regimens for human epidermal growth factor receptor 2 (HER2) negative breast tumours with positive lymph nodes or a primary node negative tumor with an unfavourable prognosis.¹¹³ Cardiotoxicity is a well-known side effect of treatment with anthracyclines. It can occur both acutely and after many years, with an increasing incidence up to fifteen years after treatment cessation.¹¹⁴ The five year incidence of chronic heart failure is 0-3.2%, depending on the specific chemotherapy regimen and the cumulative dose of anthracyclines.¹¹⁵ Furthermore, it has been suggested that anthracycline-induced cardiotoxicity (AIC) may be aggravated by the combination with taxanes.¹¹⁶ Once developed, congestive heart failure has a mortality of approximately 50% within two years.¹¹⁷

Numerous methods for the detection of AIC have been described. Although assessment of left ventricular ejection fraction (LVEF) at rest is used by most oncologists to guide treatment decisions, it is a relatively insensitive method to detect myocardial damage at an early stage.¹¹⁸ In fact, clinically evident cardiotoxicity is a late manifestation of progressive subclinical myocardial damage.¹¹⁹ Detection of subclinical cardiotoxicity would be a more desirable approach, since early detection could have clinical implications for individual patients. Treatment with cardioprotective medication, such as angiotensin converting enzyme inhibitors (ACEi), has shown to incite a potent and long-lasting recovery.¹¹⁷ Besides, studies using dexrazoxane, beta blockers, statins or ACEi as prophylactic measure against cardiotoxicity show a great reduction in cardiac events for all individual agents.¹²⁰ These treatment options underline the importance of surveillance and timely detection of any cardiac dysfunction during and after potentially cardiotoxic cancer treatment. Therefore, diagnostic tools that are able to detect myocardial damage in earlier stages are urgently needed.

Previous studies mainly focused on (NT-pro) brain natriuretic peptide (BNP) and troponins as biomarkers for AIC.^{111, 112, 121-123} However, several other biomarkers can be useful, because they play a role in the pathophysiology of AIC and general adaptive mechanisms in heart failure. Therefore, the current study aimed to investigate a diverse biomarker panel representing this complex pathophysiology. As such, tumour necrosis factor α (TNF- α , causes left ventricular dilatation through activation of matrix metalloproteinases and leads to apoptosis), troponin I (TnI, marker of myocyte injury), interleukin 6 (IL-6, induces a hypertrophic response in myocytes), NT-proBNP (marker of neurohormonal activation and released during hemodynamic stress), soluble fms-like tyrosine kinase 1 (sFlt-1, marker of vascular remodeling), ST2 (marker of myocyte stress) and galectin-3 (marker of fibrosis) were assessed.^{60, 124, 125}

Methods

An observational single centre cohort study was performed in patients who had been treated with TAC one year before inclusion in the study. The interval of one year was chosen because fatigue may be a serious problem during the first year after treatment, which may be difficult to differentiate from (early) symptoms of heart failure. All measurements and blood sampling were performed on the same day.

Patient eligibility criteria

From October 2010 to May 2012 all female patients with breast cancer, ≥ 18 years of age at the time of breast cancer diagnosis who completed (neo) adjuvant treatment with six courses of TAC one year before inclusion were asked to participate in the study. Docetaxel was given 75 mg/m², doxorubicin 50 mg/m² and cyclophosphamide 500 mg/m² in each cycle. Exclusion criteria were: evidence of breast cancer recurrence or metastatic disease, current heart disease, renal failure at the time of cardiac evaluation, diabetes mellitus, pregnancy or lactation, ionizing radiation within one year prior to inclusion, Parkinson's disease or evidence of an *m*IBG-accumulating tumour. Written informed consent was obtained from all patients. The study was approved by the Medical Ethical Committee of the Radboud University Nijmegen Medical Centre and conforms to the principles outlined in the Declaration of Helsinki.

Medical history and physical examination

The medical history and physical examination, performed in every patient, concentrated on signs and symptoms of heart failure as well as risk factors for cardiovascular disease. The past medical and drug history were recorded.

Electrocardiography

A standard 12-lead electrocardiogram was performed and analyzed, focusing on signs of left ventricular hypertrophy, ischemia and arrhythmia.

Echocardiography

We performed a transthoracic echocardiography in rest in every patient in order to compare the biomarkers to the current standard of cardiotoxicity detection methods (e.g. LVEF measurement). Echocardiography images were recorded using a commercially available system (GE Healthcare Vivid E9). LVEF was obtained from the apical four- and two-chamber views according to Simpson's rule and was considered abnormal if $< 55\%$. Next to left ventricular systolic function, left ventricular dimensions (interventricular septal thickness in diastole (IVSd), left ventricular posterior wall dimensions in diastole (LVPWd), left ventricular internal dimension in diastole (LVIDd) and left ventricular internal dimension in systole (LVIDs)), as well as left ventricular diastolic function (early to late atrial mitral

Doppler peak flow velocity ratio, E/A ratio) were measured. Left ventricular mass (LVM) was calculated as suggested by the American Society of Echocardiography.⁸⁶

Biomarkers

36 mL blood was drawn from every patient for determination of blood and serum markers. Blood samples were frozen at -80°C without any freeze-thaw cycles until assays were performed. NT-proBNP, TNF- α , galectin-3, IL-6, TnI, ST2 and sFlt-1 were assessed for primary analysis. All biomarkers were assessed on serum, except NT-proBNP and TnI, which were assessed on lithium-heparin plasma. Biochemical risk factors for cardiovascular disease (cholesterol, triglycerides, HDL, LDL, glucose, HbA1C) were determined as background parameters.

NT-proBNP and TnI were determined using the immulite 2500 chemiluminescence immunoassay system (Siemens Medical Solution Diagnostics, Deerfield, IL, USA). Age- and gender-specific reference intervals for NT-proBNP were used as established by Hess et al.⁹⁴ The limit of detection for NT-proBNP was 10 pg/mL and the coefficient of variation (CV) 3.70%. TnI was considered abnormal if detectable, with a limit of detection of 0.1 ng/mL and a CV of 3.67%. IL-6, TNF- α , sFlt-1, ST2 and galectin-3 were measured using an enzyme-linked immunosorbent assay (ELISA) following the manufacturers protocol. For IL-6 and TNF- α , the Pelikine human ELISA compact kits (Sanquin, Amsterdam, the Netherlands) were used. The standard curve ranges were 0–80 pg/mL and 0–1000 pg/mL, respectively. The mean calculated zero signal plus 3SD were respectively 0.3 pg/mL and 1 pg/mL. The CVs were 6.34% and 2.42%. For sFLT-1 ELISA's (eBioscience, Vienna, Austria) the standard curve range was 0–10 ng/mL, the mean calculated zero signal plus 2SD was 0.03 ng/mL and the CV was 5.1%. For ST2 ELISA's (R&D systems, Abingdon, UK) the standard curve range was 0–2000 pg/mL, the mean calculated zero signal plus 2SD was 5.1 pg/mL and the CV was 6.13%. For galectin-3 ELISA's (BG Medicine, Waltham, MA, USA) the standard curve range was 0–10 ng/mL, the mean calculated zero signal plus 2SD was 1.32 ng/mL and the CV was 10.4%.

Statistical Analysis

Patient characteristics were summarized for the entire study population using standard descriptive statistics. Patient data was reported as mean $\pm$ SD. Biomarker levels were reported as median (25th, 75th percentile). Abnormal levels of biomarkers according to preset standards were used as a measure for subclinical cardiotoxicity. Primary analysis consisted of determination of the prevalence of abnormal biomarkers. Pearson's correlations were used to determine the correlation between the different biomarkers and LVEF. Statistical tests were based on a two-sided significance level and the level of significance was set at 0.05.

The results of the conventional echocardiographic parameters were expressed as mean and SD. The data were studied in two subgroups of normal and abnormal NT-proBNP

levels. Relations between NT-proBNP subgroups and conventional echocardiographic parameters were studied, using the independent t-test. Statistical analysis was performed, using SPSS version 18 for Windows (SPSS Inc., Chicago, IL, USA).

Sample size considerations

The prevalence of subclinical cardiotoxicity after TAC chemotherapy is not known. In paediatric oncology however, subclinical cardiac abnormalities after treatment with doxorubicin can be detected in up to 65% of the patients and up to 5-10% may develop clinical heart failure.¹²⁶ Given that the incidence of overt heart failure after TAC is approximately 3% ¹¹⁵ for a sensitive biomarker to potentially qualify as an early predictor of cardiotoxicity, it is expected to be detectable in at least 15% of asymptomatic patients. If we would have found less than 10% we would have rejected the techniques as an ineffective diagnostic tool with respect to subclinical heart failure. Applying the minimax Simon design with alpha = 0.05, power = 0.90, H0: $p < 0.10$, Ha: $p \geq 0.25$ resulted in an initial inclusion of 31 patients. If subclinical toxicity in no more than three patients (out of 31 subjects) would have been found, the study would have terminated. If not, accrual would continue to a total of 55 patients.

Results

Fifty-five female patients were included in the study. Mean age at time of enrolment was 52.8 ±8.5 years. 52 of 55 patients (94.5%) were treated with full dose TAC and three patients had a dose reduction. One patient completed five cycles only, because of withdrawal after severe nausea and mucositis. Two patients received a docetaxel dose of 75% in the sixth cycle because of grade II peripheral neuropathy. Further patient characteristics are shown in table 3.1. Prevalence of medical history and cardiovascular risk factors are in line with the Dutch population.¹²⁷ None of the patients showed clinical signs or symptoms of heart failure. Two patients had an abnormal ECG: one patient had a pre-existent left bundle branch block, the other patient showed slow R-wave progression over the precordial leads and aspecific repolarisation disturbances.

Median biomarker levels are listed in table 3.2. Abnormal biomarker levels were detected in 29.1% of all patients. NT-proBNP was abnormal in 18.2% of all patients and significantly correlated with LVEF. TNF-α was abnormal in 7.3% of patients, but these four patients showed neither a diminished ejection fraction nor an elevated NT-proBNP. Galectin-3 was abnormal in 7.3%; in one of these four patients a diminished EF was found and in one an elevated NT-pro-BNP. IL-6, TnI, ST2 and sFlt-1 values were not elevated in any patient. Echocardiographic parameters were obtained from all patients and listed in table 3.3 by their NT-proBNP value. The mean LVEF of the study population was 62.3% (SD 7.2%). In four

Table 3.1 Patient characteristics (N = 55).

	N (%)
Age	
18-50 years	20 (36.4)
50-60 years	24 (34.6)
60-70 years	11 (20)
Tumour side	
Left	26 (46.3)
Right	22 (40)
Both	7 (12.7)
Chemotherapy	
Neo-adjuvant	6 (10.9)
Adjuvant	49 (89.1)
Type of surgery	
Mastectomy	28 (50.9)
Double mastectomy	4 (7.3)
Lumpectomy	19 (34.5)
Double lumpectomy	2 (3.6)
Ablation and lumpectomy	1 (1.8)
No breast surgery	1 (1.8)
Radiotherapy	
No	19 (34.5)
Left side thorax	21 (38.2)
Right side thorax	18 (32.7)
Axillary	11 (20)
History and cardiovascular risk factors*	
Smoking	11 (20)
Renal function disorder	0 (0)
Liver function disorder	1 (1.8)
ECG abnormalities	2 (3.6)
Medication use	
Beta Blockers	6 (10.9)
ACE/AT-II antagonist	2 (3.6)
Diuretic	9 (16.4)
Calcium antagonist	2 (3.6)
Statin	5 (9.1)
Antidiabetic	0 (0)

Table 3.1 Patient characteristics (N = 55).

	N (%)
Background laboratory values (mean ± SD)	
Cholesterol (mmole/L)	5.08 ± 0.77
Triglycerids (mmol/L)	1.49 ± 0.64
HDL-cholesterol (mmole/L)	1.47 ± 0.26
LDL-cholesterol (mmole/L)	2.93 ± 0.69
Glucose (mmole/L)	5.96 ± 1.77
HbA1c (%)	5.68 ± 0.47
HbA1c (mmole/mole)	38.75 ± 5.17

* Renal function disorder: MDRD <60 mL/min. Liver function disorder: ALAT >1.5x upper limit = 35U/L*1.5= 53 U/L. ECG abnormalities: signs of ischemia or arrhythmia.

Table 3.2 Biomarker levels.

Biomarker	Median level (25 th , 75 th percentile)	Reference value (RV)	Patients > RV, N (%)
NT-proBNP (pg/mL)	122 (62, 199)	18-50 years: <170 50-60 years: <250 60-70 years: <300*	10 (18.2)
TNF-α (pg/mL)	<2.8 (<2.8, 2.9)	<10 pg/mL ⁺	4 (7.3)
Galectin-3 (ng/mL)	12.9 (11.2, 14.8)	17.6 ng/mL ⁺	4 (7.3)
IL-6 (pg/mL)	<3.12 (<3.12, <3.12)	<10 pg/mL ⁺	0 (0)
Troponin-I (μg/mL)	<0.20 (<0.20, <0.20)	<0.2 μg/L*	0 (0)
ST2 (ng/mL)	10.7 (8.1, 12.7)	4.9-19.9 ng/mL ⁺	0 (0)
sFlt-1 (pg/mL)	<320 (<320, <320)	Not detectable ⁺ 49.2-154.3 pg/mL [‡]	0 (0)

* RV as established by our laboratory. ⁺ RV as stated in manufacturer's protocol. [‡] RV as stated by Kong et al.¹⁴⁰

patients (7.3%) an LVEF of less than 55% was observed, of which three patients' LVEF was >50%. In one patient a LVEF of 35% was detected and this patient seemed to have a severe valvular aortic stenosis. However, this patient did not show signs of clinical heart failure and, therefore, was not excluded from the study. A significantly lower LVEF (57.8% vs. 63.1%, $p=0.032$) was observed in the subgroup of patients with an abnormal NT-proBNP.

Correlations between biomarkers and LVEF are listed in table 3.4. A statistically significant negative correlation for LVEF and NT-proBNP ($r = -0.564$, $p<0.01$) was observed (figure 3.1). No significant relation was found for LVEF and any other biomarker or for different biomarkers.

Table 3.3 Echocardiographic results.

		NT-proBNP abnormal (N=10)	NT-proBNP normal (N=45)	p-value*
LV dimensions	IVSd (cm)	0.87 ± 0.13	0.91 ± 0.14	0.42
	LVPWd (cm)	1.08 ± 0.68	0.98 ± 0.49	0.59
	LVIDd (cm)	4.73 ± 0.99	4.64 ± 0.50	0.68
	LVIDs (cm)	3.34 ± 1.05	3.26 ± 0.77	0.78
LV systolic function	LVEF (%)	57.8 ± 9.1	63.1 ± 6.4	0.03
LV diastolic function	E/A ratio	1.11 ± 0.3	1.21 ± 0.4	0.41
LV mass		71.8 ± 20.0	72.2 ± 18.7	0.95

Values are expressed as mean ± standard deviation. LV = left ventricle, IVSd = interventricular septal thickness in diastole, BSA = body surface area, LVPWd = left ventricular posterior wall dimension in diastole, LVIDd = left ventricular internal dimension in diastole, LVIDs = left ventricular internal dimension in systole, E/A ratio = early to late atrial mitral Doppler peak flow velocity ratio.

* Independent t-test.

Table 3.4 Correlations.

	LVEF	NT-proBNP	TNF-α	Galectin-3
NT-proBNP	-0.564*			
TNF-α	-0.148	-0.068		
Galectin-3	0.028	0.008	0.195	
ST2	0.027	-0.058	-0.098	0.185

Assessment by Pearson's correlation. * $p < 0.01$

Figure 3.1 Correlation for LVEF and NT-proBNP ($r=-0.564$).

Discussion

In the present study the usefulness of a diverse panel of biomarkers for the detection of subclinical AIC was investigated in a homogenous group of breast cancer patients treated with TAC. Abnormal biomarker levels were detected in 29.1% of patients. NT-proBNP was abnormal in 18.2% of patients. Of these patients, one patient had hypertension (10%), compared to ten patients with a normal NT-proBNP (21.7%). Therefore, it is unlikely that elevated NT-proBNP is secondary to hypertension. NT-proBNP was negatively correlated with LVEF. The subgroup of patients with elevated NT-proBNP showed a significantly lower LVEF than those with normal NT-proBNP levels.

Comparison to currently available literature

Our findings are consistent with results of previous studies concerning the biomarkers NT-proBNP, TnI, IL-6 and TNF- α to detect subclinical cardiotoxicity after anthracycline therapy. Galectin-3, ST2 and sFlt-1 have not been evaluated previously in relation to anthracycline treatment.

NT-proBNP as a biomarker to detect subclinical AIC has been studied in both childhood cancer patients and adults. The studies concerning childhood cancer patients are very heterogeneous with respect to tumour type and duration of follow-up. All studies, however, concluded that NT-proBNP may be a useful marker for detection of subclinical AIC.¹²⁸⁻¹³⁰ In adults, one study has been performed in one hundred patients receiving 226.1 ± 8.3 mg/m² anthracyclines. This study showed only two patients with elevated NT-proBNP levels, six months after treatment.¹²¹ Another study, concerning 26 acute leukaemia patients treated with 464.3 ± 117.5 mg/m² anthracyclines, showed elevated NT-proBNP in sixteen patients (61.5%) six months after treatment, which correlated with echocardiographic parameters.¹¹¹ Besides, Romano et al. showed that persistently elevated NT-proBNP levels during anthracycline treatment are correlated to left ventricular impairment after twelve months.¹¹² However, they did not measure NT-proBNP levels during follow-up. Two studies concerning BNP levels after anthracycline therapy showed a relation between BNP level and cardiac prognosis. In one study, three out of 27 patients receiving anthracyclines demonstrated a persistently elevated BNP level and two of them died of circulatory failure.¹²³ The second study investigated BNP levels at baseline and one month, one year and two years after anthracycline therapy. Thirteen out of 53 patients eventually developed a cardiac event. In this subgroup significantly higher BNP levels were observed one year after treatment.¹²²

In several studies in children, TnI levels have been evaluated after anthracycline therapy. Two studies including 31 and 22 patients showed normal TnI levels in all patients.^{130, 131} One study showed an abnormal TnI level in two of 29 patients.¹³² A study in adults concerning 71 anthracycline treated patients showed no elevated TnI levels after one year of follow-up.¹²² Our finding of all normal TnI levels is in line with these results, suggesting that low-sensitive TnI is not a useful marker for detection of subclinical cardiotoxicity. However, recently developed high-sensitive troponin assays are potentially able to detect subclinical cardiotoxicity and are therefore incorporated into the National Cancer Institute classification of anticancer therapy induced cardiotoxicity.^{84, 133, 134}

In a study of 31 patients treated with anthracyclines IL-6 levels increased significantly directly at the end of treatment, but declined to normal levels after six months of follow-up, while TNF- α levels did not change significantly.¹³⁵ In our study, one year after cessation of chemotherapy, all patients showed normal IL-6 levels, indicating that this is not a useful biomarker to detect subclinical cardiotoxicity early after end of therapy. However, we observed abnormal levels of TNF- α in four patients (7.3%). Taken into consideration the 65% of subclinical cardiotoxicity known from paediatric oncology patients¹²⁶ and a lack of correlation between TNF- α , LVEF and NT-proBNP, we concluded that this marker lacks sensitivity to detect subclinical cardiotoxicity.

We are the first to report on the relationship between anthracycline therapy and levels of sFlt-1, ST2 and galectin-3. We detected abnormal levels of galectin-3 in four patients (7.3%). The definite role of galectin-3 in early detection of cardiotoxicity is hard to address, as galectin-3 has not been investigated in this setting before and the relation between galectin-3 levels and cardiac prognosis must be established. Levels of sFlt-1 and ST2 were normal, so these markers are not useful for early detection of cardiotoxicity one year after treatment.

Strengths and limitations of the study

For the detection of subclinical cardiotoxicity, we selected seven biomarkers representing the diverse pathophysiology of heart failure. However, others choices could have been made. Biomarkers which might have predictive value but were not included are reactive oxygen species,¹³⁶ endothelin-1,¹³⁷ myeloperoxidase¹³⁸ and glycogen phosphorylase isoenzyme BB.¹³⁹ Next, high-sensitivity TnI could have been measured instead of TnI.

Also, repeated measurement of the specific biomarkers would have been of interest, as not only absolute values but also trends in biomarker level may have predictive value. Nevertheless, in this study we decided to start with one time point only (one year after cessation of TAC), acknowledging that if biomarkers were to be identified at this time point, a larger prospective study would be indicated, incorporating several time points before and after start of chemotherapy. However, if no abnormal biomarkers could be identified at this time point at all, it would be unlikely that these markers could serve as biomarkers at even earlier points in time. The investment of time and resources of patients and investigators in a large prospective could then be spared. In fact, our study showed that a diverse biomarker panel, representing the complex pathophysiology of heart failure, does not contribute to early detection.

Finally, newer imaging techniques, for example echocardiographic strain (rate) imaging, ¹²³I-MIBG scintigraphy or cardiac magnetic resonance imaging, might be of interest in the early detection of AIC.

Compared to current literature, we investigated biomarkers for detection of subclinical cardiotoxicity in a relatively large and homogenous group of patients. Furthermore, a more diverse biomarkers panel was used.

Conclusion

The value of a diverse biomarker panel for early detection of cardiotoxicity was assessed, representing the complex pathophysiology of heart failure. We conclude that this panel does not contribute to the early detection. Future research in this field should therefore

focus on NT-proBNP. To provide definite proof of the clinical impact of this biomarker on early detection of cardiotoxicity, the next step should be a randomized controlled intervention study, either treating or not treating patients with an elevated NT-proBNP with beta blockers and ACEi to establish whether LVEF deterioration and overt heart failure can be prevented.

Relationship of promising methods in the detection of anthracycline-induced cardiotoxicity in breast cancer patients

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Abstract

Purpose: It remains challenging to identify patients at risk for anthracycline-induced cardiotoxicity (AIC). To better understand the different risk stratifying approaches we evaluated ^{123}I -metaiodobenzylguanidine (^{123}I -mIBG) scintigraphy and its interrelationship with conventional echocardiography, 2D strain imaging and several biomarkers.

Methods: We performed ^{123}I -mIBG scintigraphy, conventional and strain echocardiography and biomarker (NT-proBNP, TNF- α , galectin-3, IL-6, troponin I, ST2 and sFlt-1) assessment in 59 breast cancer survivors one year after anthracycline treatment. Interobserver and intermethod variability was calculated on planar and SPECT ^{123}I -mIBG scintigraphy, using the heart/mediastinum (H/M) ratio and washout (WO). Pearson's r and multivariate analysis was performed to identify correlations and independent predictors of ^{123}I -mIBG scintigraphy results.

Results: Delayed planar anterior whole heart ROI (WH) H/M ratios and WO were the most robust ^{123}I -mIBG parameters. Significant correlations were observed between ^{123}I -mIBG parameters and several conventional echo parameters, global longitudinal and radial strain (GLS and GRS) and galectin-3. The highest Pearson's r was observed between delayed H/M ratio and GRS (Pearson's r 0.36, $p = 0.01$). Multivariate analysis showed that GRS was the only independent predictor of the delayed WH H/M ratio ($p = 0.023$).

Conclusion: The delayed planar H/M ratio is the most robust ^{123}I -mIBG parameter. It correlates with several conventional echocardiographic parameters, GLS, GRS and galectin-3. Of these, only GRS predicts the H/M ratio.

Introduction

Anthracyclines are widely used for (neo) adjuvant treatment of breast cancer.^{141, 142} This class of drugs is associated with cardiotoxicity.³⁹ Anthracycline-induced cardiotoxicity (AIC) can be acute, which may lead to chemotherapeutic dose reduction, but is generally reversible. However, development of chronic AIC (i.e. more than one year after therapy) is often irreversible and may have a significant impact on the overall prognosis and survival of breast cancer survivors.⁴ As treatment with anthracyclines resulted in significantly improved breast cancer survival over the past decades, the importance of (early) detection and prevention of potential side effects has increased. Most commonly, the possible deleterious effects of anthracyclines on left ventricular function are monitored by left ventricle ejection fraction (LVEF) measurement using multigated radionuclide angiography (MUGA) or (2D non contrast) echocardiography.^{39, 40} However, the reproducibility of echocardiography parameters varies and both techniques only detect LVEF changes that occur after considerable damage has been acquired.^{53, 143} An adequate technique to identify patients at risk for cardiotoxicity (i.e. before the damage occurs) is still lacking.⁴⁰

The pathophysiology of AIC is complex and not yet fully understood. Recent research has focussed on the topoisomerase-II β enzyme as the core defect mechanism, which is believed to induce cell death upon the formation of a complex with the anthracycline doxorubicin.^{10, 11} This topoisomerase-II β -doxorubicin complex induced cell death subsequently triggers a cascade of cytokine release and compensatory mechanisms, which are potential targets for early detection of AIC.⁶⁰ When myocytes die, cardiac output declines, with the release of norepinephrine (NE) in the synapse by the sympathetic nervous system as one of the first neurohumoral responses.⁴³ The release of NE in combination with a decreased presynaptic NE reuptake (i.e. NE transporter downregulation) leads to an increased concentration of NE in the synaptic cleft.^{41, 42} Meta-iodobenzylguanidine (mIBG) is an analogue of the sympathetic neurotransmitter NE. Labelling of mIBG with ^{123}I allows for scintigraphic assessment of sympathetic activity and may provide a measure for early detection of AIC.⁴¹ The most commonly used methods to quantify myocardial ^{123}I -mIBG uptake are the measurement of the heart-to-mediastinum (H/M) ratio and washout (WO). Increased sympathetic cardiac activity is characterized by a decreased H/M ratio and an increased myocardial washout of ^{123}I -mIBG.

Other novel methods that may detect AIC in an early stage include 2D strain (rate) imaging with echocardiography and blood biomarkers. 2D strain (rate) imaging measures the relative deformation (i.e. stretch) of cardiac tissue in three different axes.⁵⁴ Since strain can differentiate active from passive movement, subtle regional differences can be detected long before LVEF deteriorates.⁵⁶ Blood biomarkers can be measured to provide information on cardiac pathological processes, as summarized in the cytokine hypothesis by Braunwald

et al.⁶⁰ Myocyte injury, whether it is due to hemodynamic or ischemic stress, induces release of the compensatory prohormone N-terminal pro brain natriuretic peptide (NT-proBNP), the myofibrillar protein troponin I (TnI) and different cytokines including tumour necrosis factor alpha (TNF- α) and interleukin-6 (IL-6). Subsequently, activated monocytes secrete the interleukin-1 receptor family member ST2, while macrophages produce galectin-3.⁶⁰ Furthermore, the angiogenesis promoter soluble Fms-like tyrosine kinase receptor 1 (sFlt-1) is activated.¹⁴⁴

As ^{123}I -mIBG scintigraphy, 2D strain (rate) imaging and blood biomarkers reflect different aspects of the same pathophysiological mechanism, they most likely show some inter-relationship. However, knowledge on this possible correlation is still lacking. Therefore, the aim of the current study was to study the relation between ^{123}I -mIBG scintigraphy, echocardiographic (strain) imaging, and a selection of the most promising biomarkers. We aimed to study this in a homogenous group of breast cancer survivors one year after a potentially cardiotoxic chemotherapeutic regimen containing anthracyclines.

Materials and methods

Patient selection

All adult female patients presented between October 2010 and May 2012 with breast cancer and at least one year after completion of (neo) adjuvant treatment with docetaxel, doxorubicin (i.e. anthracycline) and cyclophosphamide (TAC) were asked to participate in the study. Exclusion criteria consisted of major heart disease (i.e. myocardial infarction, percutaneous coronary intervention or coronary artery bypass graft) at the time of breast cancer diagnosis, renal failure at the time of cardiac evaluation, evidence of breast cancer recurrence or metastatic disease, pregnancy or breast feeding, participation in a research protocol with ionizing radiation one year prior to inclusion, diabetes mellitus, Parkinson's disease or an ^{123}I -mIBG accumulating tumour.

A detailed medical history and physical examination was obtained in all patients, with special attention to risk factors and signs and symptoms of cardiac disease. Current medication use was noted. A standard 12-lead electrocardiogram was performed and analysed for signs of cardiac disease and rhythm disturbances. The study was approved by the medical ethics committee of the Radboud University Medical Centre (Nijmegen, the Netherlands) and informed consent was obtained from all patients.

^{123}I -mIBG scintigraphy

Patient medication interfering with ^{123}I -mIBG uptake was interrupted for at least five half-lives after consultation of the attending physician. Thyroid ^{123}I -uptake was blocked by oral administration of 400 mg potassium perchlorate one hour before intravenous

injection of 185 MBq ^{123}I -mIBG (AdreView, GE Healthcare). Patients rested for thirty minutes prior to injection.

Planar ^{123}I -mIBG images were acquired in anterior and posterior view fifteen minutes ('early') and four hours ('delayed') after injection. Imaging was performed during ten minutes using a 20% energy window centred on the 159 keV photopeak of ^{123}I , and acquired with a medium energy collimator and stored in a 128x128 matrix. No scatter correction was applied. Subsequently, a single photon emission computed tomography (SPECT) of the thorax was performed 35 and 260 minutes post injection, obtaining 32 frames of 60 sec/frame, on a dual-head detector system, using a rotation of 180°.

Measurements on planar images

On the anterior planar images ten to twenty pixel regions of interest (ROI) were drawn over the upper mediastinum by two observers (BB, ASL) (Figure 4.1).^{46, 145} This ROI was then

Figure 4.1 H/M ratio ROI placement.

Standardized approach for the placement of the mediastinal and heart ROIs for H/M ratio determination, adapted from Somsen and Flotats.^{46, 145} Notice the upper and lower boundary defining the upper mediastinum and the mediastinal midline. The heart ROI either consists of a circular ROI including the left ventricle and the cavum (Whole heart ROI; WH) or a small circular ROI on the left ventricle lateral wall (Small LV ROI; Sm).

placed over the LV anterior wall to obtain the Small left ventricular (Sm) ROI. Furthermore, a whole heart (WH) ROI was manually determined. Both ROIs were mirrored on the posterior planar images. For all planar images, H/M ratios were calculated by dividing the cardiac average counts per pixel by the mediastinal average counts per pixel. Furthermore, a geometric mean of the heart and mediastinum counts was calculated (by means of the formula $\sqrt{\text{counts}_{\text{anterior}} \times \text{counts}_{\text{posterior}}}$), resulting in the Geo H/M ratio. Eventually, this resulted in two measurements of the H/M ratios (i.e. anterior and geometric mean) on two time points (i.e. early and delayed), and with two ROI methods (i.e. WH and Sm).

The (background corrected) myocardial WO was defined as described by Veltman.¹⁴⁶ This was done for both the anterior images and the geometric mean method, using both the WH and Sm ROI delineation method. WO is expressed as a percentage.

Single photon emission computed tomography (SPECT) images

For SPECT image interpretation, tomographic slices were reconstructed in short axis, horizontal axis and vertical long axis planes. Early and delayed images were identically aligned so that simultaneous analysis of the image planes was allowed. Mediastinal, WH and LV (i.e. including or excluding the cavum) voxels of interest (VOIs) were visually drawn using Inveon Research Workplace 4.1 (IRW, Siemens Molecular Imaging). The mediastinal VOI had a fixed spherical shape of twenty voxels and care was taken to exclude thyroid tissue. H/M ratios and WO were calculated with mean voxel count as described above.

Echocardiography and biomarkers

Methods on the measurement of conventional and strain echocardiography parameters and biomarkers have been described extensively elsewhere.^{147, 148} The current study focused on the interrelationship of these parameters with ¹²³I-mIBG values. The parameters on conventional echocardiography that were studied included the internal dimensions of the left ventricle at end-diastole (LVIDd) and end-systole (LVIDs), the posterior and septal wall thickness at end-diastole (LVPWd, IVSd), left ventricular mass (LVM), left ventricular volume at end diastole (LVEDV) and end systole (LVESV), left atrial end diastolic volume (LAEDV), left ventricular ejection fraction (LVEF), early (E) and late (A) diastolic transmitral peak flow velocity (E/A ratio), the systolic to diastolic pulmonary vein peak flow velocity (PV S/D-ratio) and early diastolic transmitral peak flow velocity (E) to early diastolic annular velocity (e') ratio (E/e' ratio). Measurements of LVEDV, LVESV, LVM and LAEDV were indexed by body surface area (BSA).

The biomarkers we studied were NT-proBNP, TNF- α , galectin-3, IL-6, TnI, ST2 and sFlt-1. Biochemical risk factors for cardiovascular disease (cholesterol, triglycerides, HDL, LDL, glucose, HbA1C) were also determined.

Statistical analysis

Patient age and time after treatment are expressed in years or months with range. Other patient characteristics are expressed in numbers of total and percentage. ¹²³I-mIBG and echocardiographic values are expressed as mean $\pm$ SD. Biochemical values are expressed as median with interquartile range.

Lin's concordance correlation coefficients (LCCs) with 95% confidence intervals are calculated for interobserver variability and depict the correlation of measurements of two different observers. For clinically relevant agreement, the following criteria are used: LCC values <0.90, 0.90-0.95, 0.95-0.99, and >0.99 were considered to indicate poor, moderate, substantial, and almost perfect agreement, respectively.¹⁴⁹ The coefficient of variation (CV) is the relative ratio of SD to mean and expressed in percentage. 95% limits of agreement in Bland-Altman plots are defined as mean $\pm$ 2SD, which is numerically expressed as the coefficient of repeatability (CR; calculated as 1.96 x SD).

Correlations of the various studied methods are expressed in Pearson's *r*. Correlations with a one-tail $p < 0.1$ were included in multivariate regression analysis, which was performed in a forward stepwise fashion. Significance was set at $p < 0.05$. All statistical analyses were performed with SPSS for Windows, version 20.0.

Results

Patient characteristics

Fifty-nine breast cancer survivors were included in the study. All patients had received a full dose of anthracyclines (300 mg/m²), except one who received 250 mg/m². None of the patients had a history of major cardiac events (i.e. myocardial infarction, percutaneous coronary intervention or coronary artery bypass graft), nor did any of them indicate chest pain or express heart failure signs/symptoms. One patient presented with a known left bundle branch block. Other patient characteristics, including risk factors and medication use are displayed in table 4.1. One year after treatment the mean LVEF was 62.6 ($\pm$ 7). LVEF was less than 55% in three patients, respectively 35, 53 and 54%. The observed LVEF of 35% was due to aortic sclerosis, which was observed on conventional echocardiography and returned to normal after surgical intervention. The other two patients did not receive follow up, since they did not meet the criteria for subclinical cardiotoxicity. None of the patients used dexrazoxane.

Planar H/M ratio and WO

Anterior and geometric mean H/M ratios and WO were obtained in all patients on both early and delayed ¹²³I-mIBG images and by both observers. All anterior H/M ratios and WO were significantly lower than geometric mean H/M ratios and WO (Table 4.2).

Table 4.1 Patient characteristics of the study group (N = 59).

	N (%)	Median (range)
Clinical characteristics		
Age in years		52 (36-69)
Time after treatment in months		12.5 (10-14)
Risk factors		
Smoking	13 (22)	
Hypertension ^a	18 (30)	
Family history ^b	9 (16)	
HbA1c >53 mmol/L	1 (2)	
BMI >30 kg/m ²	13 (22)	
Medication use		
ACEi/AT II antagonist	2 (3)	
Beta Blocker	6 (10)	
Diuretic	9 (15)	
Statin	5 (9)	
Calcium antagonist	2 (3)	
Treatment characteristics		
Tumor side		
Left	28 (46)	
Right	24 (39)	
Both	11 (12)	
Radiation left thorax side	22 (37)	
Total radiation in Gray		65 (45-142)
Cumulative anthracycline dose in mg/m ²		300 (250-300)

a Systolic blood pressure > 140 mmHg and/or use of antihypertensive medication.
b Presence of coronary artery disease in a first degree family member at <55 years in men or <65 years in women.
BMI = body mass index, ACEi = angiotensin converting enzyme inhibitor, AT = angiotensine.

Interobserver and intermethod variability

Interobserver correlations of WH H/M ratios were moderate, of Sm H/M ratios and WO poor (Table 4.2). However, mean interobserver differences were small for WH H/M ratio (Figure 4.2). Intermethod variability, describing the correlation of WH and Sm ROI definition by one observer, demonstrated poor LCCs (Figure 4.3).

Table 4.2 Planar H/M ratio and WO characteristics.

		Anterior			Geometric mean			Intermethod Δ		
		Mean		LCC	Mean		LCC	Mean		CR
		SD	CV (%)	95% CI	SD	CV (%)	95% CI	SD		
WH	Early H/M ratio	2.71	16.2	0.90	2.99	13.4	0.91	0.28 †		
	Delayed H/M ratio	2.72	18.8	0.83-0.94	2.82	15.2	0.85-0.94	0.21	0.10 †	0.41
	WO (%)	21.9	59.8	0.92	27.5	37.1	0.92	0.21	5.6 †	0.41
Sm	Early H/M ratio	13.1	2.80	0.86-0.95	10.2	15.1	0.80	5.6	11.0	
	Delayed H/M ratio	0.53	18.9	0.74-0.89	3.05	16.1	0.69-0.87	0.25 †	0.48	
	WO (%)	0.56	19.8	0.69	2.92	26.0	0.49	0.24	0.09 †	0.47
		14.0	70.0	0.53	10.7	41.1	0.40-0.70	7.9	15.5	

† p<0.001. H/M ratio = heart-to-mediastinum ratio, WO = washout, WH = whole heart ROI, Sm = small left ventricle ROI, SD = standard deviation, CV = coefficient of variation (SD/Mean), LCC = Lin's correlation coefficient, CI = confidence interval, CR = coefficient of repeatability (1.96 x SD), Δ = difference.

SPECT H/M ratio and WO

IRW-derived H/M ratios are summarised in Table 4.3. Early H/M ratios could be calculated in 52 patients (88%), delayed H/M ratios in 43 patients (73%). This discrepancy was mainly caused by technical or acquisition protocol difficulties (e.g. hardware failure, data file corruption).

Intermethod variability

The intermethod correlation of SPECT-derived WH versus LV H/M ratio (both early and late) and WO was almost perfect (LCC 0.99). Mean differences were very small (Figure 4.4).

Figure 4.2 Interobserver differences of H/M ratios.

Bland-Altman plots of interobserver difference versus mean of planar anterior (A) and geometric (B) WH H/M ratio, both on early and delayed images. Butted lines represent 95% limits of agreement. A. Mean differences: early images -0.04 (95% CI -0.10 to 0.01), $R^2 = 1.0 \times 10^{-5}$; delayed images -0.01 (95% CI -0.07 to 0.04), $R^2 = 0.01$. B. Mean differences: early images 0.03 (95% CI -0.01 to 0.08) $R^2 = 0.01$; delayed images 0.06 (95% CI 0.02 to 0.10), $R^2 = 2.2 \times 10^{-4}$.

Figure 4.2 Continued.

Table 4.3 IRW-derived ¹²³I-mIBG SPECT-indices.

	Mean (SD)	
	WH	LV
Early H/M ratio	4.28 (0.96)	4.39 (0.99)†
Delayed H/M ratio	4.35 (1.24)	4.45 (1.27)†
Washout rate (%)	20.9 (14.9)	21.5 (14.4)

† $p < 0.001$. SD = standard deviation, WH = whole heart ROI, LV = left ventricle ROI.

SPECT versus planar

The WH method is the only method used for both planar and SPECT images. Therefore, correlation between SPECT and planar H/M ratio was calculated specifically for that method. We observed a Pearson's r of 0.33 ($p < 0.05$) for the early images, $r = 0.35$ ($p < 0.05$) for the late images and $r = 0.76$ ($p < 0.001$) for WO.

Figure 4.3 The dilution effect.

Early geo WH vs. Sm H/M ratio showed an LCC of 0.87 (95% CI 0.81 to 0.91), $R^2 = 0.42$. The increasing intermethod difference is due to the dilution effect: the difference between cavum and myocardium increases when the myocardium has high ^{123}I -mIBG-uptake (i.e. the normal heart) and the ROI inclusion of blood pool (i.e. WH ROI) will account more heavily to the average heart count. Other LCCs were 0.79 (95% CI 0.71-0.86, early anterior), 0.87 (95% CI 0.81-0.91, delayed anterior) and 0.82 (95% CI 0.74 to 0.88, delayed geo). Intermethod analysis of WO showed a poor correlation of 0.82 (95% CI 0.72-0.88) for anterior images and a moderate correlation of 0.91 (95% CI 0.85-0.94) for delayed images.

Conventional and strain (rate) echocardiography and biomarker results

Although additional data on conventional echocardiographic and strain (rate) imaging and biomarkers is described in earlier work,^{147, 150} for the purpose of completeness, an overview of the obtained results is presented in Table 4.4.

Correlation of ^{123}I -mIBG with conventional and strain echocardiography and biomarkers

Significant correlations between the delayed planar WH H/M ratios and LVEDV/BSA, LVESV/BSA, IVSd, LVM/BSA, E/A ratio (conventional echocardiography), GLS, GRS (strain echocardiography) and galectin-3 (biomarker) were identified (Table 4.5). The most

Figure 4.4 IRW-derived ^{123}I -mIBG SPECT intermethod differences.

Early WH versus LV H/M ratio: mean difference 0.12, LCC 0.99 (95% CI 0.985-0.994), $R^2 = 0.20$. Delayed WH versus LV H/M ratio: mean difference 0.10, LCC 0.996 (95% CI 0.993-0.997), $R^2 = 0.15$. Butted lines represent 95% limits of agreement. The differences can be accounted for by the dilution effect. WO mean difference -0.5%, LCC 0.993 (95% CI 0.987-0.996), not included in figure.

significant correlation was observed for GRS (Pearson's r 0.36, $p=0.01$), the least significant for E/A ratio (Pearson's r 0.19, $p=0.08$). LVEDV/BSA, LVESV/BSA, IVSd, LVM/BSA, GLS and galectin-3 showed an inverse correlation, while E/A ratio and GRS showed a direct correlation.

Multivariate analysis

Since delayed anterior WH H/M ratio seems to be the most robust measurement in our population, we aimed to identify by which parameters it was influenced. Multivariate analysis showed that GRS was the only independent predictor of the WH H/M ratio (standardized $\beta=0.36$, $p=0.023$).

Table 4.4 Conventional and strain (rate) echocardiography and biomarker results.

	Study population		Reference value (RV)	Abnormal values in N (%) [*]	
	N	Mean (SD)		< RV	> RV
Conventional echocardiography					
EF (%)	59	62.6 (7.1)	≥55	4 (7)	N/A
LVEDV/BSA (ml/m ²)	58	46.2 (10.0)	35-75	7 (12)	1 (2)
LVESV/BSA (ml/m ²)	58	18.0 (6.3)	12-30	9 (16)	1 (2)
LVIDd (cm)	59	4.6 (0.5)	3.9-5.3	3 (5)	7 (12)
IVSd (cm)	59	0.9 (0.1)	0.6-0.9	0	14 (24)
LVPWd (cm)	59	0.9 (0.1)	0.6-0.9	0	15 (25)
LVM/BSA (g/m ²)	59	77 (17)	44-88	0	12 (20)
LAEDV/BSA (ml/m ²) [*]	57	22 (6)	22 (±6)	1 (2)	2 (4)
E/e' ratio	52	6.5 (2.3)	< 8	N/A	10 (19)
E/A ratio	58	1.2 (0.3)	1.3 (±0.3)	3 (6)	3 (6)
PV S/D ratio	54	1.4 (0.4)	1.2 (±0.2)	3 (6)	15 (28)
2D strain (rate) echocardiography					
GLS	57	-17.7 (3.1)	-17.8 (2.1)	2 (4)	6 (11)
GLSR	57	-0.87 (0.2)	-0.87 (0.1)	3 (5)	2 (4)
GRS	42	38.0 (10.0)	40.5 (11.4)	1 (2)	0
GRSR	41	1.45 (0.4)	2.20 (0.6)	0	0
GCS	42	-18.9 (4.6)	-20.3 (2.6)	3 (7)	7 (17)
GCSR	41	-1.03 (0.2)	-1.72 (0.3)	0	24 (57)

	Study population		Reference value (RV)	Abnormal values*	
	N	Mean (SD)		< RV	> RV
	Median (interquartile range)				
Biomarkers					
NT-proBNP (pg/ml)	59	119 (127)	18-50 years: <170 50-60 years: <250 60-70 years: <300 [†]	N/A	10 (17)
Troponin I (µg/l)	59	<0.2 (<0.2)	<0.2 [†]	N/A	0 (0)
TNF-α (pg/ml)	55	<2.8 (1.5)	<10 [†]	N/A	4 (7)
Galectin-3 (ng/ml)	55	12.9 (3.6)	<17.6 [‡]	N/A	4 (7)
IL-6 (pg/ml)	55	<3.12 (<3.12)	<10 [†]	N/A	0 (0)
ST2 (ng/ml)	55	10.6 (14.4)	4.9-19.9 [‡]	0 (0)	0 (0)
sFlt-1 (pg/ml)	55	<320 (<320)	Not detectable [‡]	N/A	0 (0)

^{*} Abnormal value is outside 2 SD when reference is defined as a mean ±SD. [†]RV as established by the Radboudumc chemical laboratory. [‡] RV as stated in manufacturer's protocol.
EF= ejection fraction, LVEDV = left ventricular end-diastolic volume, BSA = body surface area, LVESV = left ventricular end-systolic volume, LVIDd = left ventricle internal dimension at diastole, IVSd = interventricular septum at diastole, LVPWd = left ventricular posterior wall at diastole, LVM = left ventricular mass, LAEDV = left atrial end-diastolic volume, E/e' ratio = early diastolic transmitral peak flow velocity (E) to early diastolic annular velocity (e') ratio, E/A ratio = early (E) and late (A) diastolic transmitral peak flow velocity ratio, S/D ratio = systolic to diastolic ratio, GLS = global longitudinal strain, GLSR = global longitudinal strain rate, GRS = global radial strain, GRSR = global radial strain rate, GCS = global circumferential strain, GCSR = global circumferential strain rate, N/A = not applicable.

Table 4.5 Overview of interrelationship of different parameters.

		¹²³ I-mIBG scintigraphy			Conventional echo					
		Early H/M ratio*	Delayed H/M ratio*	WO*	EF	LVEDV	LVESV	IVSd	LVM	E/A ratio
¹²³ I-mIBG scintigraphy	Delayed H/M ratio*	0.80								
	WO*	-0.08	-0.53							
Conventional echo	EF	-0.12	0.034	-0.28						
	LVEDV	-0.18	-0.34	0.23	-0.34					
	LVESV	-0.08	-0.24	0.27	-0.75	0.82				
	IVSd	-0.23	-0.27	0.19	-0.04	0.09	0.05			
	LVM	-0.18	-0.23	0.23	-0.22	0.19	0.29	0.58		
	E/A ratio	0.15	-0.19	-0.17	0.03	-0.24	-0.19	-0.16	-0.28	
Strain echo	GLS	-0.09	-0.23	0.33	-0.39	0.10	0.35	-0.03	0.40	-0.44
	GLSR	-0.01	-0.11	0.19	-0.39	0.18	0.38	-0.13	0.25	0.05
	GRS	0.23	0.36	-0.19	0.24	-0.39	-0.42	-0.17	-0.49	0.49
	GRSR	-0.09	0.07	-0.04	-0.06	-0.11	-0.05	-0.03	-0.34	0.12
	GCS	-0.04	-0.03	-0.11	-0.36	0.23	0.38	-0.31	0.09	-0.24
	GCSR	0.02	-0.16	0.12	-0.30	0.25	0.35	-0.18	0.28	-0.16
Biomarkers	NT-proBNP	-0.00	-0.16	0.23	-0.56	0.38	0.65	0.12	0.36	-0.17
	TNF-alfa	0.19	0.16	0.04	-0.14	0.21	0.20	-0.14	0.07	-0.08
	Galectin-3	-0.13	-0.30	0.09	0.03	0.17	0.06	0.15	0.09	-0.32
	ST2	-0.01	-0.05	0.01	0.03	-0.22	-0.18	-0.06	-0.11	-0.10

* Planar anterior WH region. Green: $p < 0.01$; Blue: $p < 0.05$. H/M ratio = heart-to-mediastinum ratio, WO = washout, EF = ejection fraction, GLS = global longitudinal strain, GLSR = global longitudinal strain rate, GRS = global radial strain, GRSR = global radial strain rate, GCS = global circumferential strain, GCSR = global circumferential strain rate, LVEDV = left ventricular end-diastolic volume, LVESV = left ventricular end-systolic volume, IVSd = interventricular septum at diastole, LVM = left ventricular mass, E/A ratio = early (E) and late (A) diastolic transmitral peak flow velocity ratio. LVEDV, LVESV and LVM are indexed by body surface area. Troponin, IL-6 and sFlt-1 have not been tested because there were no abnormal results.

		Strain echo						Biomarkers		
		GLS	GLSR	GRS	GRSR	GCS	GCSR	NT-pro BNP	TNF-alfa	Galectin-3
¹²³ I-mIBG scintigraphy	Delayed H/M ratio*									
	WO*									
Conventional echo	EF									
	LVEDV									
	LVESV									
	IVSd									
	LVM									
	E/A ratio									
Strain echo	GLS									
	GLSR	0.65								
	GRS	-0.36	-0.17							
	GRSR	-0.11	-0.15	0.37						
	GCS	0.31	0.17	-0.18	-0.05					
	GCSR	0.35	0.44	-0.33	-0.61	0.51				
Biomarkers	NT-proBNP	0.44	0.38	-0.25	-0.08	0.41	0.42			
	TNF-alfa	0.03	-0.16	-0.05	-0.18	0.09	0.07	-0.07		
	Galectin-3	0.11	-0.05	-0.14	0.04	0.08	0.12	0.01	0.20	
	ST2	0.16	0.11	-0.13	-0.27	0.21	0.33	-0.06	-0.10	0.19

Discussion

In the current study we examined the interrelationship of an extensive panel of potential parameters for the early detection of chronic AIC, in a homogenous group of breast cancer survivors one year after treatment. There was a significant correlation between the delayed planar WH H/M ratio and several conventional echocardiographic values, GLS, GRS and galectin-3. Furthermore, GRS was identified as an independent predictor of the late planar WH H/M ratio.

Because high reproducibility is an important requirement for any diagnostic modality, we first evaluated the interobserver and intermethod variability of different methods of H/M ratio calculation to identify the most robust one, since the delineation method is a main factor hampering widespread clinical use of cardiac ^{123}I -mIBG scintigraphy.^{45, 146, 151} In most clinical studies, the WH ROI on anterior planar images is used.^{47, 48, 50, 152} We showed that indices based on the geometric mean based did not improve reproducibility, nor was there a difference between early and delayed indices. Although a Sm ROI reduces a possible 'dilution effect' (Figure 4.3), it is inferior to the WH ROI because of its high observer variability (Table 4.2). The Sm ROI should therefore not be used.

The addition of SPECT to ^{123}I -mIBG scintigraphy might increase the diagnostic potential of this technique. Although most studies focus on regional sympathetic innervation rather than a global SPECT assessment, Chen et al. described a high reproducibility and accuracy of global ^{123}I -mIBG SPECT evaluation, which allows to separate heart failure patients from healthy controls.¹⁵³ We observed a high correlation between planar and SPECT-derived parameters, especially for WO. Furthermore, SPECT H/M ratios were systematically higher than planar ratios, which is most likely caused by an overestimation of background activity on planar images.¹⁵³ Although SPECT reconstructions result in a more accurate calculation of the H/M ratio, SPECT image acquisition and reconstruction is time-consuming and performed with different protocols, yielding divergent H/M ratio ranges, thus hindering standardization.¹⁵³ Hence, global ^{123}I -mIBG SPECT imaging, although promising, does not provide sufficient added value to be recommended for use in daily clinical practice.

Several conventional echocardiographic parameters, GLS, GRS and galectin-3 showed a correlation with WH H/M ratio, but only GRS proved to be an independent predictor. GRS measures the relative deformation of the cardiac left ventricular wall in the radial direction (i.e. LV wall thickening), yielding a positive strain value during systole. In AIC, functioning myocytes are replaced by non-contracting fibrotic cells,¹⁵⁴ which leads to impaired cardiac thickening and a decrease in GRS. Due to the sympathetic response to myocardial damage, the H/M ratio will also decrease. Therefore, theoretically one would expect a direct relationship between GRS and WH H/M ratio, which was confirmed by

the results of our study. Since GLS measures shortening of the myocardial wall in the longitudinal axis, it is defined as a negative value and an inverse relationship with WH H/M ratio is expected. The results of our study indeed demonstrate an inverse correlation between GLS and WH H/M ratio, although not strong enough to predict the WH H/M ratio. No correlation, however, was observed between GCS and WH H/M ratio.

Of the studied biomarkers, only the novel blood biomarker galectin-3 showed a significant correlation with the WH H/M ratio. Galectin-3 is a protein expressed by macrophages and believed to be a mediator of the profibrotic pathway, stimulating cardiac fibroblasts to proliferate and deposit collagen.¹⁵⁵ galectin-3 concentrations are elevated in patients with acute HF and predict an adverse outcome.¹⁵⁶ In a recent study by Ky et al., no association between cardiotoxicity and galectin-3 was found, although follow-up only lasted six months.¹⁵⁵ Other studies on this issue have not been performed in adults, but a recent study in paediatric patients showed an (non-significantly) increased level of galectin-3 at least two years after anthracycline treatment.¹⁵⁷

Conventional echocardiographic parameters that correlated with the WH H/M ratio included LVEDV/BSA, LVESV/BSA, IVSd, LVM/BSA and E/A ratio. The correlation of the WH H/M ratio and LVEDV, LVESV, IVSd and LVM displayed an inverse nature, which means that both cardiac volumes and cardiac wall diameters increase as the WH H/M ratio drops. This is an interesting finding, since typical AIC in adults presents as a dilated cardiomyopathy, featuring increased ventricle sizes and thin walls.¹⁰⁰ A possible explanation for this increased wall diameter is a compensatory myocyte hypertrophy in the dilated heart. This pattern has been described before in childhood cancer survivors.^{101, 157} Another interesting finding is that the relative deformation (represented by the GRS and GLS) decreases, while the cardiac wall diameter increases. This probably reflects the replacement of active myocytes by passive (fibrotic) tissue and concurrent myocyte hypertrophy. Furthermore, we observed a (weak) direct correlation of the E/A ratio with the WH H/M ratio, indicating a concurrent decrease of the E/A ratio with the WH H/M ratio. The E-velocity indicates diastolic filling, which decreases gradually in normal subjects. The A-velocity reflects the active atrial contraction just before end diastole and normally becomes more important in elderly patients, resulting in an E/A ratio approaching one.¹⁵⁸ However, a decreased E/A ratio could also imply diastolic dysfunction, indicating AIC, although opinions on the usefulness of the E/A ratio differ.^{159, 160}

The main limitation of the current study is the lack of baseline and follow-up measurements, so we could not assess the change of parameters over time. However, the aim of the study was not to detect a change in certain parameters, nor to predict AIC, but to assess the interrelationship of different interesting parameters in the pathophysiological process of AIC.

Although the correlations of certain parameters have been studied, for example strain (rate) imaging and TnI or conventional echocardiography and strain (rate) imaging, they have not been studied for their correlation with ^{123}I -mIBG parameters. Furthermore, we focused on a homogenous group of breast carcinoma survivors with potential AIC damage, which is a patient group that has not yet been studied properly.

With the current study we have identified the relationship of the WH H/M ratio with 2D strain imaging, biomarkers and conventional echocardiography one year after anthracycline-based chemotherapy. This sheds some light on the complex pathophysiology of AIC, enabling future studies to identify appropriate parameters for the detection of AIC.

Conclusions

Delayed planar WH H/M ratio is the most robust ^{123}I -mIBG parameter. It is correlated with several conventional echocardiographic parameters, GLS, GRS and galectin-3. Of these, only GRS is an independent predictor of the WH H/M ratio. Future studies should concentrate on a combination of ^{123}I -mIBG scintigraphy, MUGA, echocardiographic strain, CMR and biomarkers, preferably in a prospective multicentre trial with long-term follow-up in breast cancer survivors.

Does diastolic dysfunction precede systolic dysfunction in trastuzumab-induced cardiotoxicity? Assessment with multigated radionuclide angiography (MUGA)

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Abstract

Purpose: Trastuzumab is successfully used for the treatment of human epidermal receptor 2 (HER2) positive breast cancer. Because of its association with cardiotoxicity, left ventricle ejection fraction (LVEF) is monitored by multigated radionuclide ventriculography (MUGA), though this is a relatively late measure of cardiac function. Diastolic dysfunction (DD) is believed to be an early predictor of cardiac impairment. We evaluate the merit of MUGA-derived diastolic function parameters in the early detection of trastuzumab-induced cardiotoxicity (TIC).

Methods: Seventy-seven trastuzumab treated patients with normal baseline systolic and diastolic function were retrospectively selected. All serial MUGA examinations were reanalysed for systolic and diastolic function parameters.

Results: Thirty-six patients (47%) developed systolic dysfunction (SD) and 45 patients (58%) DD during treatment. Both systolic and diastolic parameters significantly decreased. Of the patients with SD 24 (67%) also developed DD. DD developed prior to systolic impairment in 54% of cases, in 42% vice versa, while time to occurrence did not differ significantly ($p = 0.52$). This also applied to the subgroup of advanced stage breast cancer patients ($p = 0.1$).

Conclusion: Trastuzumab-induced SD and DD can be detected by MUGA. An impairment of MUGA-derived diastolic parameters does not occur prior to SD and therefore cannot be used as earlier predictors of TIC.

Introduction

Trastuzumab is a recombinant humanized monoclonal antibody successfully used for the treatment of human epidermal growth factor receptor 2 (HER2) positive breast cancer. It prolongs the survival in metastatic disease and, when combined with adjuvant chemotherapy, also improves outcome in early-stage breast cancer.¹⁶¹ However, its use is associated with an increased risk of cardiotoxicity, which frequently leads to temporary or definite discontinuation of trastuzumab therapy.

The overall incidence of trastuzumab-induced cardiotoxicity (TIC) varies according to the definition used, but ranges from 2% to 7% for trastuzumab monotherapy, 2% to 13% for trastuzumab combined with paclitaxel, and can be as high as 27% when used in combination with anthracyclines.²³ TIC is often transient after discontinuation of trastuzumab, but may be irreversible if detected in a late stage.^{162, 163} Cardiotoxicity may be symptomatic with signs and symptoms of congestive heart failure, but in the vast majority, it is asymptomatic. If treatment with trastuzumab is continued despite a decrease in cardiac function, severe and even irreversible loss of cardiac function may occur. Thus, close cardiac monitoring in patients undergoing trastuzumab therapy is of paramount importance.

Evaluation of the left ventricular systolic function through measurement of the left ventricular ejection fraction (LVEF), using multigated radionuclide angiography (MUGA) or echocardiography, is the accepted method in assessing cardiac function during trastuzumab treatment. However, deterioration of LVEF only becomes apparent if the myocardium has lost its considerable functional reserve, and therefore is a relatively late expression of left ventricular dysfunction.⁶³ The detection of changes in cardiac parameters preceding decrease in LVEF allows the identification of patients who might benefit from early cardio-protective measures. Consequently, the likelihood of discontinuation of effective systemic treatment, potentially compromising the patients' long-term outcome, might be markedly reduced.

It has been shown that diastolic impairment of the left ventricle occurs before deterioration in LVEF in anthracycline-induced cardiotoxicity (AIC).^{164, 165} For TIC, Lange et al. and Does et al. echocardiographically studied diastolic dysfunction (DD) in relatively small groups, but their results contradict.^{166, 167} Cochet et al. evaluated MUGA-derived diastolic parameters and found no significant impairment of diastolic function during trastuzumab therapy, but they included only early stage breast cancer patients, used a short follow-up period of one year and did not study the time interval of the first impairment of diastolic function.⁶³

MUGA remains a reference technique for the evaluation of LVEF because of its standardized and reproducible outcome. Apart from routine use for LVEF determination, a MUGA study

can provide diastolic parameters. Peak filling rate (PFR) and time to peak filling rate (TPFR) derived from the LV time-activity curve, are the commonly used diastolic parameters. A decrease and/or delay in these parameters are typical disturbances of a normal filling pattern. The lower limit of PFR is 2.50 end-diastolic volumes per second (EDV/s). The TPFR is expected to be less than 180 ms in a normal subject.^{168, 169} Studies have demonstrated that diagnosing diastolic abnormalities by a low PFR on MUGA correlates well with changes seen on Doppler echocardiography.¹⁷⁰

We hypothesized that a change in diastolic function parameters measured by MUGA precedes deterioration in LVEF. Therefore, we evaluated MUGA-derived diastolic and systolic function parameters in breast cancer patients treated with trastuzumab, in order to determine whether diastolic function parameters can be used as early detectors of TIC.

Methods

Patient population

HER2 positive female breast cancer patients, treated with trastuzumab from 2003 to 2011 in the Radboud University Medical Centre Nijmegen and with a MUGA examination before trastuzumab, were retrospectively selected from registered data (N = 105). Both women with metastatic disease and early stage breast cancer were included. Patients with a prior history of severe cardiac events or an LVEF <50% were excluded from trastuzumab therapy (N = 1). Patients with abnormal baseline PFR or TPFR values (N = 27) were excluded from our primary analysis, but evaluated in a separate subgroup analysis. The remaining study population consisted of 77 subjects. Of these patients the history of MUGA scans and cardiac disease (until January 2015) was collected and analyzed.

Treatment regimens

After surgical staging, patients were selected for locoregional therapy and adjuvant treatment. In the non-metastatic setting, patients received adjuvant chemotherapy with doxorubicin, cyclophosphamide, a taxane and trastuzumab. In the metastatic setting, patients received several chemotherapeutic regimens, consisting of anthracyclines, taxanes, bevacizumab, capecitabine, CMF (cyclophosphamide, methotrexate, fluorouracil) and FEC (fluorouracil, epirubicin, cyclophosphamide) in divergent order, but all patients received trastuzumab during a certain time span. Trastuzumab was administered in a standard regimen: first dose at 8 mg/kg followed by a three-week schedule of 6 mg/kg diluted in 250 ml of sodium chloride 0.9%. When SD was observed, trastuzumab treatment was ceased or postponed until LVEF was recovered.

Multigated Radionuclide Angiography

All subjects underwent cardiac monitoring by serial MUGA examinations at our institution. The baseline MUGA was obtained before the start of treatment, with an every three-month assessment during therapy. The final examination was acquired one year after completion of trastuzumab or earlier in cases where discontinuation of therapy was indicated. MUGA examinations were performed in supine position, with multiple gated ECG-triggered sampling after injection of 740 MBq (20 mCi) 99mTc radiolabelled red blood cells and with acquisition from the left anterior oblique (LAO) view. 32 frames per cardiac cycle were used. The R-R interval and the heart rate (beats/min) were recorded. Cardiac cycles with an R-R interval outside 20% of the median were rejected. Acquisition lasted till six hundred accepted cycles were included.

MUGA recordings were reanalysed for systolic and diastolic function parameters. The reference parameter for systolic function was LVEF, while reference parameters for diastolic left ventricular function were PFR and TPFR. LVEF was calculated from the time-activity curve; PFR and TPFR were acquired from the first derivation of the time-activity curve after cycle-dependent background correction, by a clinically valid software program (Hermes Medical Solutions, Stockholm). Cardiac systolic dysfunction (SD) was defined as a LVEF <50% or a drop of ten or more absolute points in baseline LVEF.¹⁷¹ DD was defined as a PFR <2.5 EDV/s or a TPFR >180 ms.^{63, 169}

Statistical analysis

For all individuals, a time registration (in days) was made from baseline MUGA up to the last examination after treatment. Time intervals to first SD or DD were calculated. If DD is an early detector of TIC, the time interval to first impairment of diastolic parameters should be smaller than the time interval to first impairment of systolic parameters. To test these intervals the following convention was used: if no impairment of systolic parameters was seen, the time to SD was set to the follow-up time. The same was done if no DD was observed. A comparison of time intervals of the two parameters was made using the Wilcoxon signed rank test.

In-time differences of LVEF, PFR and TPFR were tested for significance with the paired t-test and a Holms correction for multiple testing was done.¹⁷² For the in-time assessment of these parameters the first six MUGA examinations were used. The level of significance was set at 0.05. All calculations were made with SPSS 20.0 for Windows.

Results

Demographic and clinical characteristics of the study population are displayed in Table 5.1. The number of MUGA scans performed ranged from two to nineteen per patient (median six, $N = 46$). The number varied with disease stage and the patient's tolerance of trastuzumab (i.e. decrease of LVEF). In patients with early stage breast cancer trastuzumab was administered for one year, corresponding with seventeen cycles of 21 days. Generally, patients with metastatic disease continued trastuzumab therapy longer and underwent more MUGA examinations. Those with poor tolerance of trastuzumab and the possible need for therapy withdrawal were monitored more frequently, with an increasing number of scans and shorter time intervals. Necessity of additional scans was based on clinical grounds and at the physician's discretion. In the vast majority, MUGA examinations were performed in a three-monthly interval during therapy. Follow-up of symptoms indicating cardiac disease ranged from 33 to 117 months. One patient developed clinical symptoms of cardiac failure during therapy and follow-up. This patient showed both DD and SD on MUGA, respectively after 62 and 129 days. None of the other patients received additional cardiac intervention during trastuzumab treatment or follow-up.

Of the 77 subjects included in our study, SD at any point in time during or after treatment was detected in 36 patients (47%, Table 5.2). Mean LVEF significantly decreased in time (Figure 5.1), with a mean LVEF of 58.6% at MUGA 6. Of these 36, eleven (31%) still showed SD at the last MUGA. 45 patients (58%) showed DD at any time point, of which 39 patients showed a PFR of <2.5 EDV/s and 26 showed a TPFR >180 ms. Nineteen patients had both abnormal PFR and TPFR. Both parameters significantly decreased in time (Figure 5.1), with a mean PFR of 2.8 EDV/s and a mean TPFR of 148 ms at MUGA 6. DD was present in 29 of 45 patients (64%) at the last MUGA.

Of the 36 patients with LVEF values below normal, 24 (67%) showed also deterioration of PFR or TPFR. Of these 24 patients, who were considered to have both SD and DD, thirteen patients (54%) showed DD prior to SD and ten patients (42%) vice versa. Five of thirteen patients recovered to baseline systolic function. In one patient a simultaneous decrease in systolic and diastolic function was observed. Furthermore, in 21 of 77 patients (27%) DD occurred without SD, while SD without DD occurred in twelve patients (16%).

In the subgroup of 24 patients, the median time to the first decrease was 122 days (range 36-581) for SD and 121 days (range 9-672) for DD, which was not significantly different (Wilcoxon signed rank test, $p = 0.56$). Comparison of time intervals between the first moment of SD and DD in all patients, using the Wilcoxon signed rank test, did not show any significant difference in both parameters ($p = 0.10$, Figure 5.2).

Table 5.1 Clinical characteristics of study population with normal ($N = 77$) and abnormal ($N = 27$) diastolic function at baseline.

	Normal diastolic function		Abnormal diastolic function		<i>p</i>
	N (%)	Mean (SD)	N (%)	Mean (SD)	
Clinical data					
Age (years)		50.3 (10.4)		55.4 (7.8)	<0.05
BMI (kg/m ²)		25.2 (4.4)		27.8 (5.2)	<0.05
Cardiovascular risk factors					
Hypertension	11 (14)		10 (37)		
Diabetes	3 (4)		1 (4)		
Smoking	12 (16)		6 (22)		
Use of AT blockers	3 (4)		5 (19)		
Use of beta blockers	7 (9)		11 (41)		
Cancer characteristics					
Early stage breast cancer	64 (83)		16 (59)		
Metastatic breast cancer	13 (17)		11 (41)		
Location					
Right breast	31 (40)		11 (41)		
Left breast	44 (57)		13 (48)		
Both sides	2 (3)		3 (11)		
History of (neo)adjuvant anthracyclines	63 (82)		20 (74)		
Left side radiation therapy	27 (35)		7 (26)		
MUGA data (baseline)					
General					
Heart rate (beat/min)		76 (10.7)		76 (14)	NS
R-R interval (ms)		801 (104)		820 (161)	NS
Systolic function					
LVEF (%)		63.6 (6.6)		59.9 (6.0)	<0.05
PER (EDV/s)		3.55 (0.59)		3.32 (0.52)	NS
TPER (ms)		133 (20)		145 (25)	<0.05
Diastolic function					
PFR (EDV/s)		3.42 (0.74)		2.74 (0.66)	<0.01
TPFR (ms)		137 (17)		190 (44)	<0.01

BMI = body mass index, AT = angiotensin, MUGA = multigated radionuclide angiography, LVEF = left ventricular ejection fraction, PER = peak ejection rate, EDV = end diastolic volume, TPER = time to peak ejection rate, PFR = peak filling rate, TPFR = time to peak filling rate, NS = not significant.

Table 5.2 Frequencies of SD and DD in the total population (N = 77).

	N (%)
SD	36 (47)
DD	45 (58)
PFR <2.5 EDV/s	39 (87)
TPFR >180 ms	26 (58)
Abnormal PFR + TPFR	19 (25)
DD + SD	24 (31)
DD before SD	13 (54)
DD after or concurrent with SD	11 (46)

SD = systolic dysfunction: LVEF <50% or a drop of 10 or more absolute points in baseline LVEF.
DD = diastolic dysfunction: PFR <2.5 EDV/s or TPFR >180 ms.
PFR = peak filling rate, TPFR = time to peak filling rate.

Figure 5.1 MUGA trends in time.

Serial results of left ventricle ejection fraction (LVEF, **A**), peak filling rate (PFR, **B**) and time to peak filling rate (TPFR, **C**) at six time points, T1 depicting baseline scan and T2 - T6 consecutive MUGA scans. Number of evaluated MUGA scans: T1 = 77, T2 = 74, T3 = 72, T4 = 63, T5 = 46 and T6 = 46. In general, MUGA examinations are acquired every three months, but can be made earlier at physicians' discretion. Individual time points could therefore deviate from population mean. All values are mean with 95% confidence interval.

Figure 5.1 Continued.

Subgroup analyses

Next, we performed a subgroup analysis of patients with early stage and advanced stage (i.e. metastatic) breast cancer, since the latter group may have received trastuzumab over a longer period of time, whereas patients with early breast cancer were treated during a fixed period of twelve months (Table 5.3). In both patient groups there was no significant difference detected.

Figure 5.2 Wilcoxon signed rank test.

Visualization of Wilcoxon signed rank test showing DD before SD in 34 cases and SD before DD in 22 cases. In the other 21 cases, both events were simultaneous or did not even occur. There was no significant difference in both groups ($p = 0.09$).

To evaluate anthracycline influence, another subgroup analysis was done in the small subgroup of anthracycline naïve patients ($N = 14$, Table 5.3). In this group we detected a significant difference in time to DD compared to SD ($p = 0.02$).

Additionally, to assess radiation therapy influence, a subgroup analysis was performed in left-sided radiotherapy naïve patients ($N = 50$). Although DD developed before SD (median time 111 versus 133 days), this was not significant according to the Wilcoxon signed rank test ($p = 0.15$, Table 5.3).

Subgroup analysis of patients with baseline DD

Twenty-seven patients were excluded from the primary analysis due to DD at baseline. Of these, ten patients had a PFR < 2.5 EDV/s, twelve a TPFR > 180 ms and five both. Patient characteristics are displayed in Table 5.1. Besides a significant difference in diastolic parameters, age, BMI, LVEF and PER differed from the initial patient group (Table 5.1).

Seventeen patients (63%) developed SD in a median time of 116 days (range 28-1206). Mean LVEF, however, did not significantly change in the course of trastuzumab use ($p = 0.08$). Also, diastolic function remained constant (PFR $p = 0.14$, TPFR $p = 0.75$).

Table 5.3 SD and DD in patient subgroups.

		Breast cancer stage		Anthracycline use		Left-sided radiation therapy	
		Early (N = 64)	Adv. (N = 13)	Naïve (N = 14)	Treated (N = 63)	Naïve (N = 50)	Treated (N = 27)
SD	N (%)	29 (45)	7 (54)	6 (43)	30 (48)	21 (42)	15 (56)
	Median, days (range)	129 (36-581)	138 (49-407)	150 (49-407)	122 (36-581)	133 (36-538)	129 (62-581)
DD	N (%)	34 (53)	11 (85)	12 (86)	33 (52)	28 (56)	17 (63)
	Median, days (range)	149 (17-672)	136 (9-573)	111 (9-573)	157 (18-672)	111 (18-575)	156 (9-663)
SD vs. DD*, p		0.26	0.12	0.02*	0.51	0.15	0.39

* by Wilcoxon signed rank test.

SD = systolic dysfunction: LVEF $< 50\%$ or a drop of 10 or more absolute points in baseline LVEF.

DD = diastolic dysfunction: PFR < 2.5 EDV/s or TPFR > 180 ms.

Adv. = advanced, PFR = peak filling rate, TPFR = time to peak filling rate.

Discussion

Since subclinical signs of cardiac dysfunction are supposed to predict the development of future heart failure, many parameters of diastolic and systolic function have been proposed to detect early cardiotoxicity.³⁹ However, conclusive evidence on the use of the most optimal and meaningful combination of cardiac function parameters is still lacking.

The present study retrospectively investigated whether impairment of systolic function is preceded by MUGA-detectable DD in a group of 77 female breast cancer patients undergoing trastuzumab therapy. The results of this study, showed a nearly even number of patients with DD preceding SD (54%), as compared to the number of patients with the opposite order (42%). However, in 27% of patients DD occurred without SD, while in 16% SD was observed without deterioration of diastolic function. Furthermore, in the relatively small subgroup of advanced breast cancer patients up to 85% showed a decrease in diastolic function, in contrast with 54% SD. The time interval in which the patients developed either one did not differ significantly. The rates of DD and SD in the small subgroup of anthracycline naïve patients were 86% and 43% respectively, with significantly different time intervals. For radiation therapy naïve patients the differences were small (SD 42% and DD 56%). In general, both LVEF and diastolic parameters significantly decreased

due to trastuzumab admission (Figure 5.1). Lastly, in the subgroup of patients that suffered from baseline DD, 63% also developed SD, but there was no significant decrease in systolic or diastolic function.

Diastolic impairment is presumed to be an early predictor of developing heart failure, thought to develop before systolic impairment in AIC,^{63, 64, 164, 173} while this is not yet studied in TIC. In AIC, the endocardium is most susceptible to the deleterious effects of anthracyclines, resulting in interstitial fibrosis, while in the same stage of disease the mid-myocardial fiber layers are not yet affected.⁶⁵ In TIC, the main pathophysiologic mechanism is inhibition of HER2 in cardiomyocyte tissue. The HER2-pathway is required for cell survival and continuing function and seems to be stimulated in situations of myocardial stress, such as anthracycline treatment.²⁶ HER2 inhibition results in depletion of ATP and subsequent dysfunction of contractility.²⁷ For TIC, in contrast to AIC, it has been shown that it is not dose dependent, is often transient after drug discontinuation and can be safely re-administered after recovery of LVEF.²⁶

The findings of the present study are supported by the results of Lange et al.¹⁶⁶ and Does et al.¹⁶⁷ and are also in agreement with a previous report on short-term anthracycline-based chemotherapy, showing simultaneous impairment of left ventricular systolic and diastolic radionuclide parameters.¹⁷⁴ Interestingly, although developed simultaneously, in our population we found that 31% and 64% of patients showed persistent SD and DD at their last MUGA scan. However, only one patient developed symptomatic heart failure. This patient showed persistent DD, but recovered systolic function on the last MUGA.

Reduced diastolic (time to) peak filling rates are thought to detect early chemotherapy-induced cardiotoxicity with greater sensitivity, but to date the additional value of these parameters in the prediction of future heart failure, compared to serial MUGA-based assessment of LVEF, has not been proven.¹⁷⁵ Our study evaluated the same parameters in a trastuzumab-treated patient population and resulted in comparable findings. It is the first study, however, that evaluates the time to occurrence and includes patients with metastatic disease in the evaluation.

In general, nearly all patients that do not have metastases at diagnosis are treated with anthracyclines. Consequently, it is expected that subgroups of anthracycline naive and advanced stage patients are generally the same and display a (nearly) identical cardiac dysfunction pattern. Interestingly, we found a significant shorter time interval to DD in the anthracycline naive subgroup, while subgroup analyses of early versus advanced (i.e. with metastases) stage did not show such a difference. However, the number of patients in both subgroup analyses is small and therefore no conclusions can be made based on this observation.

Reproducibility is very important for any imaging method. In a recent review the inter- and intraobserver reproducibility of MUGA-derived LVEF turned out to be near perfect.⁵³ The reproducibility of PFR and TPFR was already known to be excellent.¹⁷⁶

Several other modalities in the early detection of cardiotoxicity have been studied, although they mainly focus on AIC. The two most promising cardiac serum biomarkers are (high-sensitivity) TnI and N-terminal brain natriuretic peptide (NT-proBNP). A study in 251 women receiving trastuzumab identified TnI as a predictor of cardiotoxicity risk, but all patients had a history of anthracycline treatment. It is therefore not known whether increased TnI reflected AIC catalysed by trastuzumab, or true TIC.¹⁷⁴

Other imaging modalities such as echocardiographic strain (rate) imaging are thought to track early changes in cardiac function by measuring the relative deformation of myocardial segments and describing the contraction/relaxation pattern of the heart.^{147, 175} Although only few studies assess the use of echocardiographic strain (rate) imaging in TIC, these suggest that several strain-derived parameters can be used as independent early predictors for TIC.^{99, 177, 178}

¹²³Iodine-*meta*-iodobenzylguanidine (¹²³I-*m*IBG) is an ¹²³I labelled norepinephrine analogue, used to noninvasively assess the sympathetic myocardial innervation. Because ¹²³I-*m*IBG is not metabolised, it deflects neuronal integrity and eventually cardiac status.⁴² To date, there is no experience in the use of ¹²³I-*m*IBG for the evaluation of TIC, except for a small study in nine patients with a confirmed LVEF decrease, that suggests that risk stratification and monitoring in this group is possible.¹⁷⁹

Cardiac magnetic resonance imaging (CMR) has been studied in the assessment of TIC. The main finding in TIC is delayed enhancement of the LV lateral wall in the mid-myocardial segment.^{177, 180} In AIC, T1-weighted hyperenhancement within three days of the first administration is associated with reduced LVEF on day 28 after chemotherapy. Threshold values predicting LVEF impairment are not yet known. Although CMR is expensive, time-consuming and less widely available, its great accuracy and reproducibility will render CMR an important cardiotoxicity imaging modality in the near future.²⁷

Study limitations

In our study group prior treatment with anthracyclines, radiotherapy and/or concomitant heart failure therapy (angiotensin blockers and beta blockers) could have influenced the overall incidence of SD and DD. This, however, adequately reflects the clinical situation and gives the study a higher relevancy and applicability. Above that, our aim was to detect a difference in the time interval to SD or DD, not to estimate the overall incidence of

cardiac dysfunction. All the primary study subjects had normal systolic and diastolic baseline values and heart failure therapy was hardly used at baseline (Table 5.1).

We did not observe a statistical significant difference in time to SD and DD between early and advanced stage breast cancer patients or between patients that were treated with or naïve to left-sided radiation therapy. This could be due to the small size of the subgroups. However, we did observe a significant difference in patients treated with or naïve to anthracyclines, which is expected to be even more significant if more patients had been included. Therefore, we think this reflects a relevant finding of our subgroup analysis.

In our institution cardiotoxicity is defined as an absolute drop of ten percent-points in LVEF or a decrease below 50% in patients with baseline LVEF >50%, thereby reducing the chance of false positive test results. However, more recent and commonly used guidelines define cardiotoxicity as a reduction of LVEF of $\geq 5\%$ to $<55\%$ with symptoms of heart failure or an asymptomatic reduction of the LVEF of $\geq 10\%$ to $<55\%$.¹⁸¹ Rates of SD may differ according to the definition used, impeding inter-study comparison. The definition used in our study may have led to an underestimation of SD in our population, however, without affecting study outcome.

Despite this, we found a high rate of SD in our study population (47%), as well as an even higher rate of DD (58%), which is discordant with reported literature figures.²³ There are several possible explanations for this. First, this could be due to the fact that patients with metastatic disease, with a generally longer duration of treatment and thereby longer exposure to the deleterious effects on cardiac function, were also included in our study. Especially the rate of DD in the advanced stage breast cancer patient group was markedly higher than in the early stage breast cancer group (85% vs. 53%). However, rates of SD in both groups were only slightly different; 45% in the group with early stage breast cancer, and 54% in the group with metastatic disease. Another explanation for the high incidence of SD could be that due to the retrospective and clinical nature of the study, patients with suboptimal cardiac function are still included for trastuzumab treatment, while these patients might have been excluded in a prospective trial. Consequently, our group reflects a realistic group of breast cancer survivors and is not biased towards optimal cardiac function. Finally, in our institution MUGA examinations were done in a three-month fashion, while in other institutions this period might be longer and minor changes in cardiac function are not detected.

Conclusion

The results of the present study show that both trastuzumab-induced SD and DD can be detected by MUGA, but that there is no certain order of occurrence. Therefore, MUGA-derived diastolic parameters seem not suitable as earlier predictors of TIC. Newer promising imaging strategies in the early screening for potential cardiotoxicity have been proposed. Further clarification about the predictive value of these techniques is much awaited.

Catecholamines influence myocardial ^{123}I -*meta*-iodobenzylguanidine uptake in neuroblastoma patients

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Abstract

Purpose: Cardiac ^{123}I -*meta*-iodobenzylguanidine (^{123}I -mIBG) imaging can be influenced by several factors. We evaluated the relationship between catecholamine measurements and cardiac ^{123}I -mIBG uptake in neuroblastoma patients.

Methods: Thirty neuroblastoma patients were retrospectively assessed on cardiac ^{123}I -mIBG uptake and urinary catecholamine dopamine and metabolites, homovanillic acid (HVA) and vanillylmandelic acid (VMA). Cardiac ^{123}I -mIBG uptake was quantified by heart-to-mediastinum (H/M) ratios, which were calculated into standard deviation scores (SDS) using age-specific reference values.

Results: In seventeen (57%) and twelve patients (40%) H/M ratio measurements were below -1.0 and -2.0 SDS at diagnosis. A significant inverse correlation between the average of urine metabolites HVA and VMA, and H/M ratio SDS was observed ($r = -0.39$, $p = 0.04$). Furthermore, there was a significant correlation between the urinary catecholamine metabolite HVA and H/M ratio SDS ($r = -0.40$, $p = 0.04$).

Conclusion: Routine calculation of H/M ratios in ^{123}I -mIBG scintigraphy of neuroblastoma patients is not helpful because it will not identify cardiac ventricular dysfunction in this patient category. A low H/M ratio on ^{123}I -mIBG scintigraphy is explained by increased catecholamine levels secreted by neuroblastoma tumours.

Introduction

Radioiodinated Iodine-123-*meta*-iodobenzylguanidine (^{123}I -mIBG) is a structural analogue of the neurotransmitter norepinephrine (NE) and is taken up and stored in human tissue by the NE transporter.¹⁸² ^{123}I -mIBG shows a high uptake in both normal sympathetically innervated tissues, such as the heart, and in adrenergic tumours that highly express neuronal NE transporters, such as neuroblastoma.

^{123}I -mIBG scintigraphy has proven to be a powerful tool in the detection, staging and follow-up of childhood neuroblastoma.^{183, 184} Nowadays, ^{123}I -mIBG imaging is also used to assess cardiac sympathetic innervation, which can be helpful in identification and follow-up of cardiac dysfunction in heart failure.^{38, 185} As neuroblastoma patients are at risk for cancer treatment induced cardiotoxicity with heart failure as a possible result, assessing cardiac uptake of ^{123}I -mIBG in patients undergoing ^{123}I -mIBG scintigraphy as part of the tumour staging and follow-up could be of potential importance.

However, exact measurement of myocardial ^{123}I -mIBG uptake is essential and can be disturbed by several external or patient related factors.⁴⁵ Factors that can influence myocardial ^{123}I -mIBG uptake are scanner settings, patient's age,¹⁸⁶ use of specific medication¹⁸⁷ and elevated levels of circulating catecholamines. The relationship between myocardial ^{123}I -mIBG uptake and circulating catecholamines has been shown in adult patients with pheochromocytoma.^{188, 189} However, data correlating cardiac ^{123}I -mIBG uptake and catecholamine levels in neuroblastoma patients are limited and do not include adjustment for age-specific changes in normal patients.¹⁸⁹

Recently, reference values for cardiac ^{123}I -mIBG uptake in children were published.¹⁸⁶ Therefore, the aim of the study was to determine myocardial ^{123}I -mIBG uptake in children with newly diagnosed neuroblastoma and to examine whether cardiac ^{123}I -mIBG uptake, independent of age, is influenced by circulating catecholamines.

Material and methods

Patients

All paediatric patients diagnosed with neuroblastoma at our institution between January 2000 and December 2011 were included in the present retrospective analysis. The detection of neuroblastoma was based on ^{123}I -mIBG scintigraphy accompanied by raised concentrations of urinary catecholamine dopamine and metabolites, homovanillic acid (HVA) and vanillylmandelic acid (VMA). Diagnosis of neuroblastoma was confirmed by the presence of characteristic histopathological features of tumour tissue in all children. All

patients were treated according to the Children's Oncology Group protocols for medium and high-risk neuroblastoma.¹⁹⁰ Neuroblastoma treatment protocols comprise the same elements all over the world: induction chemotherapy, surgery, high dose chemotherapy and irradiation if necessary. The cumulative dosage of doxorubicin, the chemotherapeutic drug with potential cardiotoxic side effects, remained unchanged in the optimised treatment protocols during the years of the study period.

¹²³I-mIBG scintigraphy

All ¹²³I-mIBG scintigraphic tests were performed before treatment. None of the patients received any medication that would interfere with myocardial ¹²³I-mIBG uptake. Since 2009, for ¹²³I-mIBG scanning doses were calculated from the paediatric dose card, based on a nonlinear weight-dependent increase, with a minimum dose of 80 MBq.¹⁹¹ Before the year 2009 a standardised dose was given dependent of age: <3 years 75 MBq, 3-15 years 150 MBq. Carrier-added ¹²³I-mIBG for mIBG imaging was used in all patients and was received from Covidien plc., subsidiary Mallinckrodt Medical B.V., Petten, The Netherlands (specific activity of 148 MBq/mg) and from GE Healthcare B.V., Eindhoven, The Netherlands (specific activity of 0.46-4.6 GBq/mg). The tracer was injected intravenously after a thirty minute rest period. Anterior planar images of the whole body, chest and abdomen (with a 128x128 matrix) were acquired at four and 24 hours post-injection (p.i.) on dual-head gamma cameras (Siemens: E.cam Gantry Dual Head, E.cam Gantry Signature Series Dual Head and E.cam extended Gantry Dual Head v3). A medium-energy all purpose, high resolution, parallel hole collimator with a 15% window centered at 159 keV was used. Throughout the eleven years, the same collimator was used and also the acquisition time has been constant. Myocardial uptake of ¹²³I-mIBG was determined from both four and 24 hours p.i. images. To quantify myocardial ¹²³I-mIBG uptake, the heart-to-upper mediastinum uptake (H/M) ratio was calculated.¹⁹² Therefore a small, fixed thirty-pixel region of interest (ROI) was placed along the left ventricular anterior wall (LVAW) and in the upper mediastinum according to the method described by Chen et al. (Figure 6.1).¹⁸⁶ Total counts per ROI were obtained and the LVAW H/M ratio was calculated by the formula

$\frac{LVAW_{total\ counts}}{Mediastinum_{total\ counts}}$. The H/M ratios were created independently by two observers

(B.B., L.G.). They were blinded to clinical patient information. The average value of the two H/M ratio measurements was used in the study. The H/M ratios at 24 hours p.i. were converted to standard deviation scores (SDS) based on age-related reference H/M ratio values.¹⁸⁶

The formula for calculating the SDS: $\left(\frac{H/M\ ratio_{average} - H/M\ ratio_{mean\ reference\ population}}{standard\ deviation_{reference\ population}} \right)$.

Figure 6.1 Region of interest (ROI) drawing from a cardiac ¹²³I-mIBG planar anterior image.

ROI is placed along the left ventricular anterior wall (1, red) and mediastinum (2, yellow).

Urine analysis

Urinary levels of dopamine and metabolites HVA and VMA were measured before treatment and were expressed as a ratio to creatinine excretion (μmol/mmol creatinine). Dopamine, HVA and VMA were assayed by high-performance liquid chromatography with electrochemical detection.^{193, 194} Creatinine was measured by the Jaffé method.

Echocardiography

To register potential cardiotoxic side effects of anthracyclines as part of the chemotherapeutic treatment for neuroblastoma, all patients underwent routine transthoracic echocardiographic study in left lateral position prior to treatment. Echocardiographic measurements of left ventricular end systolic (LVESD) and end diastolic diameter (LVEDD) and left ventricular fractional shortening were determined. The fractional shortening

percentage (FS%) was calculated using the following formula: $\frac{(LVEDD - LVESD)}{LVEDD} \times 100$. To compare

left ventricular dimensions within the study group, LVESD and LVEDD were adjusted for age and body size and converted into SDS by using a reference population of normal controls.¹⁹⁵

Standard deviation scores were calculated as follows: $SDS = \left(\frac{value_{measured} - value_{mean\ of\ controls}}{standard\ deviation_{controls}} \right)$.

Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD), unless indicated otherwise. The distribution of urinary catecholamine metabolites was assessed for normality using q-q plots and Shapiro-Wilk test. The interobserver agreement of the H/M ratio measurements by the two observers was calculated with an intraclass correlation coefficient. The linear dependency of urinary dopamine, HVA and VMA on the H/M ratios was assessed by a linear regression analysis. The Spearman rank correlation was used to correlate urinary catecholamine metabolites with H/M ratio SDS. *P*-values <0.05 were considered significant for the correlation coefficient (*r*). All calculations were performed using SPSS 19.0.

Results

Between January 2000 and December 2011, thirty patients (twelve male, 40%) were diagnosed and treated for neuroblastoma in our medical centre. Median age at the time of diagnosis was two years (range: five days - seventeen years). Patients were staged according to International Neuroblastoma Staging System criteria.¹⁹⁶ Female sex was over-represented in stage 1-2 and 4S, with a male-to-female ratio in the total study group of 1:1.5. Patient characteristics at diagnosis are summarized in Table 6.1. Median administered ^{123}I -mIBG dose was 8.15 MBq/kg (range 3.2-27.0).

H/M ratio measurements showed a good interobserver reliability.¹⁹⁷ H/M ratios determined with the LVAW ROI at 24 hours p.i. were calculated into a SDS using age-specific reference H/M ratio values¹⁸⁶ and are depicted in Table 6.1. The mean H/M ratio SDS was -1.74 ± 0.28 . Twenty-seven patients (90%) showed an H/M ratio below 0 SDS. In seventeen (57%) and twelve patients (40%) values were below -1.0 and -2.0 SDS. None of the patients had pre-existing congenital cardiac disease, diminished cardiac function or dilated cardiomyopathy at presentation. Left ventricular function parameters were all within normal ranges: mean fractional shortening $36.1 \pm 6.1\%$, LVEDD SDS -0.19 ± 1.12 and LVESD SDS 0.04 ± 1.23 .

Urine catecholamine metabolites characteristics were available in 26 and 27 of thirty patients at the time of diagnosis for VMA and HVA, respectively. Most of these patients showed elevated urinary HVA and VMA levels above 5 $\mu\text{mol}/\text{mmol}$ creatinine, 96% and 89% respectively (Figure 6.2). Different patterns of urine catecholamines were observed within patients with varying HVA:VMA ratios ranging from 0.60 to 42.88 with a median of 1.16. Regression analysis, using linear dependency, showed a significant correlation between the H/M ratio SDS and the average amount of urine catecholamine metabolites HVA and VMA: $r = -0.39$, $p = 0.04$ (Figure 6.2). Age-corrected H/M ratio decreased with increasing urinary catecholamine metabolite levels. Furthermore, there was a significant

Figure 6.2 H/M ratio SDS.

In 27 patients at diagnosis, related to **A**) average of urinary HVA and VMA ($\mu\text{mol}/\text{mmol}$ creatinine), $r = -0.39$, $p = 0.04$. And **B**) urinary HVA ($\mu\text{mol}/\text{mmol}$ creatinine), $r = -0.40$, $p = 0.04$.

correlation between the H/M ratio SDS and the urinary catecholamine HVA: $r = -0.40$, $p = 0.04$ (Figure 6.3).

There was no correlation between H/M ratio SDS and the urinary catecholamine metabolite VMA ($p = 0.12$) or dopamine ($p = 0.60$), neither between H/M ratio SDS and cardiac parameters shortening fraction ($p = 0.47$), LVEDD ($p = 0.56$) and LVESD ($p = 0.18$). Other clinical patient characteristics, such as administered ^{123}I -mIBG dose (MBq/kg) ($p = 0.49$), ^{123}I -mIBG producer ($p = 0.68$), stage of disease ($p = 0.47$), gender ($p = 0.34$), MYCN gene amplification ($p = 0.87$) and ^{123}I -mIBG tumour uptake ($p = 0.81$) did not show a significant correlation with cardiac ^{123}I -mIBG uptake.

Table 6.1 Patient characteristics.

	Stage	Age (yrs)	Sex	Primary location	M+ disease	MYCN
1	1	0.5	F	Left adrenal gland	No	NA
2	2A	0.2	M	Paraspinal: abdominal	No	NA
3	2A	0.5	F	Paraspinal: sacral region	No	NA
4	2A	0.9	F	Paraspinal: abdominal	No	NA
5	2A	2.7	F	Paraspinal: thoracic	No	NA
6	2A	1.3	F	Paraspinal: abdominal ^a	No	NA
7	2B	0.6	F	Cervical	No	NA
8	3	0.8	F	Paraspinal: thoraco-abdominal ^a	No	NA
9	3	1.7	F	Paraspinal: thoracic ^a	No	NA
10	3	2.2	F	Right adrenal gland	No	NA
11	3	1.4	M	Left adrenal gland	No	A
12	3	2.7	M	Paraspinal: thoraco-abdominal ^a	No	NA
13	3	9.6	F	Intraspinal: sacral, with local invasion	No	UA
14	4	1.1	F	Left adrenal gland	Yes	NA
15 ^b	4	0.4	M	Paraspinal: abdominal	Yes	NA
16	4	1.7	M	Left adrenal gland	Yes	A
17	4	2.1	F	Paraspinal: abdominal	Yes	NA
18	4	2.4	M	Unknown primary tumor	Yes	NA
19	4	2.7	M	Left adrenal gland	Yes	A
20	4	3.5	M	Paraspinal: thoracic ^a	Yes	NA
21 ^b	4	4.0	M	Left adrenal gland	Yes	NA
22	4	4.1	F	Paraspinal: abdominal ^a	Yes	NA
23	4	4.3	M	Left adrenal gland	Yes	A
24	4	4.8	F	Paraspinal: thoraco-abdominal	Yes	NA
25 ^c	4	10.4	M	Liver hilus	Yes	NA
26	4	12.6	F	Paraspinal: abdominal	Yes	NA
27	4	17.0	M	Paraspinal: thoracic	Yes	NA
28	4S	5 d	F	Cervical	No	NA
29	4S	8 d	F	Paraspinal: abdominal	Yes	NA
30	4S	0.4	F	Paraspinal: abdominal	Yes	NA

‡ as defined by the International Neuroblastoma Risk Group; * in $\mu\text{mol}/\text{mmol}$ Creatinine; ** left ventricular anterior wall ROI 24h p.i.; ^a = extend in neural foramina; ^b = no urine catecholamines available; ^c = patient depicted in Fig. 6.3.

M+ = metastatic, MYCN = myc myelocytomatosis viral related oncogene, neuroblastoma derived, H/M ratio SDS =

	Shimada	Risk Group‡	¹²³ I-mIBG	HVA*	VMA*	H/M ratio SDS**
1	Fa	-	C	216.4	120.4	-4.42
2	Fa	-	C	21.1	27.8	-3.58
3	Fa	-	C	11.1	8.9	-1.12
4	Fa	-	G	28.0	26.0	0.36
5	Fa	-	C	8.1	3.3	-0.44
6	Fa	+/-	C	86.5	74.4	-2.66
7	Fa	-	C	23.3	25.0	-2.88
8	Fa	+/-	G	189.7	145.7	-2.11
9	Fa	+/-	C	23.3	36.7	-0.16
10	Fa	+/-	C	213.2	72.2	-0.99
11	U	+	C	34.0	6.0	-2.70
12	U	+	G	41.5	69.4	0.05
13	U	+	C	3.1	4.6	-0.95
14	Fa	+/-	C	13.0	15.2	-0.58
15 ^b	Fa	+/-	UO	NA	NA	-1.69
16	U	+	G	214.4	5.0	-1.82
17	U	+	C	300.0	130.5	-1.09
18	U	+	G	355.8	212.3	-4.86
19	U	+	UO	49.0	71.5	-0.30
20	U	+	C	8.7	NA	-0.54
21 ^b	U	+	G	NA	NA	-2.01
22	U	+	C	59.5	65.3	0.39
23	Fa	+	C	24.1	39.3	-1.86
24	U	+	C	131.7	48.0	-0.77
25 ^c	U	+	C	60.9	61.8	-3.91
26	U	+	C	101.8	34.7	-0.60
27	U	+	C	120.0	42.0	-2.05
28	Fa	-	C	270.0	124.3	-4.14
29	Fa	-	C	50.0	76.7	-4.27
30	Fa	-	C	103.0	156.0	-0.77

heart-to-mediastinum ratio standard deviation score at time of diagnosis, M = male, F = female, A = amplified, NA = not amplified, UA = unreliable assessment, Fa = favourable, U = unfavourable, C = ¹²³I-mIBG source Covidien, G = ¹²³I-mIBG source GE Healthcare, UO = ¹²³I-mIBG source of unknown origin, d = days, yrs = years.

Figure 6.3 Patient no. 25.

Influence of chemotherapy during a five year treatment period on urinary catecholamine and metabolite concentrations and cardiac ^{123}I -mIBG uptake (day 0 = start chemotherapeutic treatment). After start of chemotherapy urinary VMA and HVA levels decreased and cardiac ^{123}I -mIBG uptake increased. Two years after start of treatment the patient relapsed; urine metabolites VMA and HVA levels increased and the H/M ratio diminished. Urine metabolites VMA and HVA levels and the H/M ratio recovered after treatment for relapse.

To illustrate the influence of chemotherapeutic treatment on urinary catecholamine concentration and cardiac ^{123}I -mIBG uptake, Figure 6.4 displays the absolute H/M ratio and urinary catecholamine values over time during a five year treatment period within one patient (no. 25, Table 6.1).¹⁹⁷ High urine catecholamine metabolites with low H/M ratio were seen at diagnosis and during relapse, with an increase of H/M ratio and concomitant decrease in urinary VMA and HVA during treatment for primary and relapsed disease.

Discussion

In this study we showed a high incidence of low myocardial ^{123}I -mIBG uptake in children with newly diagnosed neuroblastoma at diagnosis (i.e. prior to treatment) after adjustment for age by using a paediatric reference group. Myocardial ^{123}I -mIBG uptake prior to treatment was less than -1.0 and -2.0 SDS in 57% and 40% of patients, respectively. In addition, the data confirm an age-independent association between cardiac ^{123}I -mIBG uptake and secreting catecholamines.

Many factors have the potential to interfere with ^{123}I -mIBG uptake and storage, which particularly should be taken into account when evaluating myocardial ^{123}I -mIBG uptake. Several studies reported that cardiac ^{123}I -mIBG uptake decreases with increasing age in children and adults.^{186, 198} Although few studies previously described a variable low cardiac ^{123}I -mIBG uptake in newly diagnosed neuroblastoma patients prior to treatment,^{188, 189} the cardiac ^{123}I -mIBG uptake has never been corrected for the age-influencing effect so far. This might have resulted in a misconception and underestimation of the incidence of low cardiac ^{123}I -mIBG uptake in untreated neuroblastoma patients. Suga et al. reported an incidence of only 23% when using a qualitative grading system of ^{123}I -mIBG uptake.¹⁸⁹ With the quantitative and age-corrected H/M ratio in the present study, a higher incidence of low myocardial ^{123}I -mIBG uptake prior to treatment was observed.

Our results suggest that poor myocardial ^{123}I -mIBG uptake is most likely caused by the presence of excessive catecholamines excreted by neuroblastoma tumour cells. The inverse correlation between cardiac ^{123}I -mIBG uptake and urinary catecholamine metabolites (average HVA and VMA) in children with neuroblastoma in this study is in accordance with previous published studies. These studies demonstrated a concomitant increase in cardiac ^{123}I -mIBG accumulation and decrease in circulating plasma catecholamines during treatment of neuroblastoma and pheochromocytoma.^{188, 189} Another study in rats showed that exogenous intravenous administration of high doses of NE led to decreased uptake of ^{123}I -mIBG in the heart.¹⁹⁹

The average of HVA and VMA was used for comparison with H/M ratio SDS because of two reasons. First, the HVA and VMA catecholamine metabolites are proven to be sensitive surrogate parameters for circulating dopamine and NE levels respectively,²⁰⁰ of which both interact with myocardial ^{123}I -mIBG uptake if present.¹⁸² Second, a great variation in the amount of urinary HVA excretion compared to VMA excretion is described within patients with neuroblastoma.^{201, 202} This phenomenon is referred as biochemical heterogeneity with respect to catecholamine production and has been attributed to differences in cell maturity between different neuroblastoma tumours.²⁰² We also found this variability in our study population, with HVA:VMA ratios ranging from 0.60 to 42.88. Some patients excreted a much greater amount of HVA compared to the amount of VMA and vice versa.

Except a statistically significant correlation between the average of HVA and VMA metabolites and H/M ratio SDS also a significant correlation was found between the catecholamine metabolite HVA and H/M ratio SDS. No significant correlation was found when catecholamine metabolite VMA was correlated to H/M ratio SDS. The biochemical heterogeneity in catecholamine production (HVA vs. VMA) within the patients may explain this absence of correlation. Furthermore, in the analysis of the relation between the average of HVA and VMA metabolites and H/M ratio SDS, there were three patients

who also demonstrated low cardiac *mIBG* uptake (H/M ratio SDS < -2) with relatively low catecholamine levels (average HVA and VMA < 25 µmol/mmol Cr), depicted in Figure 6.2. A possible explanation for this could be an inability to metabolise dopamine into HVA and NE into VMA as a result of an enzyme blockade or reduced enzyme activity, which has been described in neuroblastoma patients.^{201, 203} With this enzyme blockade, measuring the surrogate parameter HVA or VMA will give low HVA or VMA results but subsequent high dopamine and/or (nor)epinephrine values that could result in low myocardial *mIBG* uptake. Two out of three patients have a relatively high urine dopamine excretion compared to their VMA and HVA levels. However, comparison of their dopamine concentration within the study group was not possible as dopamine measurements were not routinely done in all patients because of its lower sensitivity in the detection of neuroblastoma.²⁰² In the other patient no dopamine measurement was performed at all.

The simultaneous change of urinary catecholamine metabolites and myocardial ¹²³I-*mIBG* uptake during a five year treatment course of a single patient with relapse of disease is interesting and supports our hypothesis (Figure 6.4). However, a larger cohort with a longer follow-up over time is needed to draw definite conclusions.

Several underlying mechanisms have been suggested for the catecholamine-related decrease in myocardial ¹²³I-*mIBG* uptake. First, there is a competition at the binding site of the NE transporter for neuronal uptake in the myocardial tissue between oversecreted circulating catecholamines and the circulating NE analogue ¹²³I-*mIBG*.¹⁸² Second, high catecholamine levels have demonstrated to cause a downregulation of the cell membrane NE transporter in the cardiac sympathetic neurons. Animal models have shown that chronic exposure to NE causes a reduction in myocardial uptake of ¹²³I-*mIBG* in proportion to the downregulation of NE transporter binding sites.²⁰⁴ In patients with heart failure, which is characterized by its hyperadrenergic state with increased systemic levels of NE, a reduction of cardiac NE transporter density and activity in myocardial tissue samples has been described.²⁰⁵ Third, uptake of an unequal great part of the injected ¹²³I-*mIBG* from the plasma by the neuroblastoma tumour (sequestration) could, theoretically, also reduce uptake of myocardial ¹²³I-*mIBG* in our patients. However, previous reports showed tumour sequestration of imaging agents as an insignificant contributing factor.^{206, 207} In this study, we did not find a correlation between ¹²³I-*mIBG* tumour uptake and H/M ratio.

As reported in the introduction, we explored the potential role of ¹²³I-*mIBG* scintigraphy in the detection of cancer treatment induced cardiotoxicity.^{38, 185} The results of our study, however, imply that ¹²³I-*mIBG* scintigraphy is not useful for early detection of cardiotoxicity during treatment in patients with catecholamine producing tumours. However, for identifying late-onset cardiotoxic side effects after effective oncologic treatment (i.e. in the absence of catecholamines), ¹²³I-*mIBG* scintigraphy could be a helpful complementary

tool. One should always consider the possibility of persistent cardiac dysfunction if restoration of the suppressed ¹²³I-*mIBG* uptake is insufficient after chemotherapeutic treatment.¹⁸⁹

Limitations

The study was retrospective and used H/M ratio reference values for comparison from another single centre study. Although our post-processing measurement methods were adapted to the reference study with good interobserver variability, the study of Chen et al. did not describe which image acquisition parameters were used. Comparison of these acquisition parameters was therefore not possible. It is, however, known that stringent procedure guidelines for radio-iodinated *mIBG* scintigraphy in children were defined in 1997 and have been used worldwide ever since.²⁰⁸⁻²¹⁰ The duration of being exposed to catecholamines before diagnosis, regardless of the levels, might also have had an impact on the results. However, because it is unknown from which moment catecholamines were present (perhaps before the onset of first clinical symptoms), we were not able to take this issue into account.

Conclusion

Routine calculation of H/M ratios in ¹²³I-*mIBG* scintigraphy of neuroblastoma patients is not helpful because it will not identify cardiac ventricular dysfunction in this patient category. A low H/M ratio on ¹²³I-*mIBG* scintigraphy is explained by increased catecholamine levels secreted by neuroblastoma tumours.

Letter to the editor:
Interobserver variability of
heart-to-mediastinum ratio in ^{123}I -*m*IBG
sympathetic imaging

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TO THE EDITOR: With great interest we read the article by Chen et al. regarding factors that affect heart-to-mediastinal ratio (H/M ratio) in ^{123}I -*meta*-iodobenzylguanidine (^{123}I -mIBG) cardiac sympathetic imaging.⁴⁵ The authors describe the variation of H/M ratio among institutions and the factors that contribute to this. Furthermore, they provide different protocols that could minimize this variation.

One of the significant contributors to the variation of H/M ratio in cardiac imaging, are the settings of the regions of interest (ROIs) that are used. Currently, as correctly described by Chen et al.,^{45, 186} there are three main approaches for drawing the heart ROI. One is to place the ROI over the whole heart, thereby neglecting the 'dilution' effect in patients with a dilated heart (i.e. lower blood pool activity in the cavity than the myocardium) but resulting in high reproducibility.^{44, 45} The second approach is to place the ROI along the left ventricular (LV) wall, inter alia described by Gerson et al.,²¹¹ which possibly leads to greater inter- and intra-observer bias.⁴⁵ In the third approach a smaller ROI is placed along the anterior part of the LV wall.¹⁸⁶

In our current ongoing study in paediatric patients by van der Palen et al.,²¹² we compared both approaches described by Chen (i.e. the 'whole heart ROI' and the 'small anterior wall ROI'). The reason for comparing those instead of the 'LV wall ROI' by Gerson is twofold: possible comparison of our results with Chen's references and potential inaccurate placement of an ROI along the complete LV wall in small paediatric hearts (i.e. the child's heart is often too small for exact placement over the LV wall). Several studies on paediatric cardiology also use this approach.^{69, 213}

When studying the subsequently calculated H/M ratios we noticed that the interobserver variability when using these methods of ROI placement was only minor, when H/M ratios were obtained independently by two observers at four and 24 hours post injection in our population of thirty paediatric patients. Intraclass correlation coefficients (ICCs) were as follows: for the small anterior wall ROI H/M ratio 0.882 (95% CI: 0.578-0.956) and for the whole heart ROI H/M ratio 0.955 (95% CI: 0.904-0.979) at four hours. At 24 hours, the ICC was 0.911 (95% CI: 0.716-0.965) for the small anterior wall ROI H/M ratio and 0.951 (95% CI: 0.896-0.977) for the whole heart ROI H/M ratio. Therefore, it can be concluded that the use of both ROI definition methods in children results in substantial to almost perfect interobserver agreement concerning H/M ratio. Furthermore, this suggests that whole H/M ratio has better agreement than small H/M ratio for both four and 24 hours p.i. scans. Besides, both methods also showed a high within patient ICC ratio of 0.865. This implies that inpatient variability is fairly small and both methods give almost identical results.

Although the ICC of both methods of ROI placement are rather similar, the most promising method is the whole H/M ratio four hours post injection. To reduce differences in H/M

ratios due to interobserver variability the method of ROI placement should be standardised, and it is reasonable to define this method as the gold standard for H/M ratio measurement. However, as stated in the article of Chen et al., this method could underestimate the H/M ratio in patients with a dilated heart (being the target population in the article of Chen) and a relatively high negative contribution of the myocardial cavity.⁴⁵ This, however, has no impact on our population, which does not contain patients with cardiomyopathy. Small anterior wall H/M ratios do not have this disadvantage and showed adequate intraobserver agreement.

Therefore, we can conclude that the best method to determinate the H/M ratio in cardiac sympathetic imaging depends on the patient characteristics of the study group. In paediatric patients we recommend the use of the whole heart H/M ratio, since it shows the best intraobserver agreement. With Chen we would like to emphasize the importance to standardise ROI placement methods in order to compare study results and minimise the inpatient, interpatient and interinstitutional variation.

Cardiac molecular changes during doxorubicin treatment in mice

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Abstract

Purpose: Although doxorubicin (DOX) is a potent cytotoxic drug, it possesses distinct cardiotoxic properties, of which the underlying pathologic mechanisms are not fully unravelled. It is important to elucidate the complex pathophysiology on a molecular level to allow early detection of cardiotoxicity and selection of prophylactic treatment strategies.

Methods: The cardiac distribution of ^{99m}Tc -sestamibi, ^{99m}Tc -Annexin V, ^{99m}Tc -glucaric acid and ^{18}F -FDG was evaluated using a gammacounter at baseline and after one, two, three or four cycles of 15 mg/kg DOX in healthy male BALB/c mice and controls. In addition, cardiac expression of non-imaging markers (Bcl-2, Caspase 3 and 8, TUNEL, HIF-1 α , p53) was assessed by Western Blot or immunohistochemistry and mitochondrial membrane potential was measured.

Results: A total of two hundred mice (one hundred treated, one hundred controls) was evaluated. All radiopharmaceuticals showed a significantly increased uptake compared to controls, with peak cardiac uptake after one (^{99m}Tc -Annexin V), two (^{99m}Tc -sestamibi), three (^{18}F -FDG) or four (^{99m}Tc -glucaric acid) cycles of DOX. Strong correlations ($p < 0.01$) were observed between ^{99m}Tc -Annexin V, Caspase 3 and 8 and TUNEL and between ^{18}F -FDG and HIF-1 α .

Conclusion: The cardiac response to anthracyclines at low doses starts with apoptosis depicted by ^{99m}Tc -Annexin V and confirmed by histological apoptosis markers. Late apoptotic membrane disintegration can be detected by ^{99m}Tc -sestamibi and ^{99m}Tc -glucaric acid but both have some limitations that need attention. ^{18}F -FDG uptake signifies an early adaptive response to DOX and has the highest potential to serve as a clinically exploitable early marker of reversible cardiotoxicity in the near future.

Introduction

Doxorubicin (DOX) is a glycoside antibiotic belonging to the class of anthracyclines and a highly effective cytotoxic drug used against both solid and haematological cancer types.²⁷ The counterpart of this powerful antineoplastic effect is that DOX (and anthracyclines in general) induces apoptosis in noncancerous cells, including cardiomyocytes, causing acute and chronic cardiotoxicity (termed anthracycline-induced cardiotoxicity; AIC) and significantly impact patients' outcome.⁴

Some of the key cardiotoxic effects of DOX are the inhibition of topoisomerase II β , the formation of reactive oxygen species (ROS) along with depletion of endogenous antioxidants and the subsequent triggering of the intrinsic (mitochondria-dependent) and the extrinsic (death receptor) apoptotic pathway.^{10, 214} Both lead to the activation of Caspase 3 and the subsequent cascade of events that is typical for apoptosis and can be divided into an early part (Caspase activation and phosphatidylserine (PS) expression), a midterm part (DNA fragmentation, chromatin condensation and membrane phospholipid metabolism) and a late part (membrane blebbing and cell shrinkage).²¹⁵ These mutations in cardiomyocyte homeostasis underlie the pattern of subclinical cardiotoxicity and a better understanding of the involved mechanisms offers the opportunity for multitargeted exploration of early AIC, leading to the development of noninvasive and quantitative detection tools that promote the personalised approach of patients.

Although available imaging techniques like echocardiography, multigated radionuclide angiography (MUGA) and cardiac magnetic resonance imaging (CMR) already evaluate anthracycline-induced changes in an accurate and reproducible manner, these anatomy-based parameters represent alterations that occur late in the process of AIC and provide low sensitivity in the detection of subclinical myocardial dysfunction.⁵³ With the increasing population of cancer survivors, there is a growing need to change the paradigm from the current concept of simply measuring the worsening of myocardial function to the early molecular identification of patients at risk of irreversible cardiotoxicity, thereby optimizing the revenues of protective therapy. Indeed, detection of subclinical AIC as early as possible has become an important field of medical investigation in recent years.

To further elucidate the mechanisms that are involved in the cardiotoxic process, we aimed to map the cardiomyocyte response using an animal model of DOX-induced cardiotoxicity by four different mechanistic approaches. Since early apoptosis is characterised by changes in PS expression and the activation of several intracellular regulatory proteins, PS distribution was studied with Technetium-99-*metastable* (^{99m}Tc -) Annexin V and correlated to histological apoptosis markers Caspase 3 and 8, p53, anti-apoptotic Bcl-2 and DNA fragmentation analysis. Cardiomyocyte (mitochondrial)

membrane integrity ($\Delta\psi_m$), depicting later apoptotic changes, was assessed with ^{99m}Tc -sestamibi and ^{99m}Tc -glucaric acid and correlated to JC-1 fluorescent dye, Bcl-2 and DNA fragmentation. Furthermore, perfusion changes were studied using ^{99m}Tc -sestamibi and correlated to hypoxia-inducible factor (HIF-1 α), which plays an important role in the molecular response to hypoxemic conditions.²¹⁶ Finally, glucose metabolism was evaluated by fluorine-18-fluorodeoxyglucose (^{18}F -FDG) and correlated to HIF-1 α , since the latter is suggested to activate glucose transporters (GLUT).²¹⁷ A schematic overview of the studied markers and their place in the cardiotoxic process is provided in Figure 8.1.

Materials and Methods

Study design

Groups of four healthy male BALB/c mice were treated with intraperitoneal (i.p.) administration of 15 mg/kg DOX during one, two, three or four weeks respectively, resulting in a maximum cumulative dose of 60 mg/kg, which corresponds to a potentially cardiotoxic dose of 1050 mg per cycle for a 70 kg male patient.²¹⁸ At baseline and after completion of each cycle of DOX the cardiac and biodistribution of ^{99m}Tc -sestamibi (group A), ^{99m}Tc -Annexin V (group B), ^{99m}Tc -glucaric acid (group C) and ^{18}F -FDG (group D) was investigated approximately one hour after intravenous (i.v.) tail vein administration. The control group consisted of four healthy male BALB/c mice treated at the same time points and conditions with saline administration (group E, F, G and H).

Deoxynucleotidyl transferase biotin-dUTP nick-end labelling (TUNEL) staining, cardiac expression of Bcl-2, p53, Caspase 3, Caspase 8 and HIF-1 α and $\Delta\psi_m$ were assessed by Western Blot analysis (WB), immunohistochemistry (IH) and flow cytometry (FITC) at the same time points described for radiopharmaceuticals. Figure 8.2 shows a schematic representation of the study design.

Radiopharmaceutical preparation and administration

^{99m}Tc -sestamibi

The lipophilic cation sestamibi (Cardiolite, Bristol-Myers Squibb S.r.l.) was radiolabelled by adding water to 1-3 ml of sodium pertechnetate ($^{99m}\text{TcO}_4$)Na and ten minutes of heating at 100 °C. After cooling, a monovalent lipophilic cationic complex was retrieved (^{99m}Tc -sestamibi-6-2-metossi-isobutyl-isonitril), of which on average 18.5 MBq in 0.1 mL was injected i.v.

^{99m}Tc -Annexin V

Human recombinant Annexin V (National Institute of Health, Bethesda, USA) was radio-labelled through combined in vitro incubation at room temperature for thirty minutes of

Figure 8.1 The studied cardiac markers in the process of AIC.

DOX = doxorubicin, PS = phosphatidylserine, NRG = neuregulin, GLUT = glucose transporter, ROS = reactive oxygen species.

60-90 μg di-HYNIC-Annexin V diluted in 0.5 ml of physiological saline solution, 0.74-1.11 MBq pertechnetate anion ($^{99m}\text{TcO}_4^-$), which was freshly eluted by a 6 GBq generator (Amersham, GE HealthCare), 10-20 μg of reduction agent (stannous chloride dehydrate, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) and 1.5 mg of tricin (N-[tri(hydroxymethyl)methyl]glycine) as co-ligand. Labelling yield was 85-90%. ^{99m}Tc -Annexin V was then injected in a 0.1 mL solution containing on average 18.5 MBq of ^{99m}Tc -Annexin V which corresponds to a total protein concentration of 4.5 ng per animal.

^{99m}Tc -glucaric acid

^{99m}Tc -glucaric acid was prepared from a lipophylized kit containing 12.5 mg of D-glucaric acid and ^{99m}Tc -pertechnetate in the same manner as described for Annexin V. The radiopharmaceutical was injected in 0.1 mL solution, containing on average 18.5 MBq ^{99m}Tc -glucaric acid.

Figure 8.2 Schematic study design.

Groups A to D represent the radiopharmaceuticals described in the text. Groups E to F represent controls. Nonimaging markers are WB, IH and FITC expression markers. † = sacrifice of mice.

¹⁸F-FDG

The glucose analogue ¹⁸F-FDG was provided as a bulk vial (Gluscan, GE Healthcare), from which a sample of 18.5 MBq in 0.1 ml was injected. To avoid the intake of carbohydrates, the mice that received ¹⁸F-FDG were provided a fat-enriched diet.

Biodistribution and histological analysis

After completion of their regimen and one hour after injection of the radiopharmaceuticals animals were anaesthetised and sacrificed. Samples of thyroid, kidney, tail, bone, lung, heart, muscle, blood, stomach, skin, liver, spleen, intestine and urine were taken out and weighted directly afterwards. Radioactivity of these samples was registered for 180 seconds by a gamma counter (2470 Automatic Gamma Counter Wizard, Perkin Elmer), along with an aliquot of the injective and the tail to correct for partial extravasation and expressed as percentage of injected dose per gram of tissue (%ID/g).

Before gamma-counting, two three-micrometer cardiac tissue slices were obtained for histology: one was frozen at -20°C in dry ice, the other placed in 4% formalin. After treatment with a protease inhibitor (1:100 in 10 mM Tris buffer, pH 8) apoptotic cells were assessed with direct fluorescence fragment end labelling of DNA breaks (TUNEL kit, Fluorescein FragEL, Calbiochem). Caspase 3 and HIF-1α were immunostained using anti-human-mouse active antibodies (Rabbit anti-Caspase 3 and rabbit anti-HIF Polyclonal Antibody, Chemicon International) after incubation with a fluorescent-labelled secondary antibody (Alexa Flour 488 goat anti-rabbit IgG, Molecular Probes). Cells stained with TUNEL, Caspase 3 and HIF-1α were counted in ten randomly selected high-power tumour fields (magnification x200) and expressed as percentage of total cells. Furthermore, cardiac samples were analyzed by Western Blotting, to identify the specific amount of Bcl-2, p53 (both Santa Cruz Biotechnology Inc.), Caspase 3 and Caspase 8 (both Cell Signalling Technologies Inc.).

Measurement of Δψ_m

Inner Δψ_m was measured by a single fluorescent lipophilic cation (JC-1) that marks the loss of potential. For this purpose mitochondria were isolated from myocardial tissue by a dedicated kit (Sigma-Aldrich), stained with JC-1 and analyzed by FITC (MACSQuant, Miltenyi Biotech). Red stained cells represent intact mitochondria, green stained cells disrupted mitochondria. This is then transformed to a red-to-green (i.e. intact to disrupted mitochondrial) ratio and described as an upper and lower border (UR and LR). Consequently, a decreasing ratio represents a disintegration of membrane potential.

Statistical analysis

Biodistribution and immunofluorescent data are expressed as mean and median %ID/g tissue and standard deviation (SD). Data were analysed by nonparametrical tests: Kruskal-Wallis and Dunn to evaluate the differences in groups at different time points and Mann-Whitney U to test significance between DOX-treated and DOX-naïve mice. Furthermore, Spearman's rho was used to assess correlations. Significance was set at *p*<0.05. Statistics were performed using IBM SPSS 20 for Windows.

Results

Doxorubicin treatment

A total of one hundred mice were examined, distributed over the four radiopharmaceuticals and observed at five different time points (including baseline). The mice that received up to 45 mg/kg DOX did not show any significant side effects and appeared healthy. The mice that received 60 mg/kg either died or showed significant side effects such as weight loss, oedema and alopecia. Furthermore, one hundred control mice were imaged.

Cardiac and biodistribution of imaging biomarkers

The cardiac and biodistribution of radiopharmaceuticals is shown in figures 8.3, 8.4 and 8.5 and summarised in table 8.1.

^{99m}Tc-sestamibi

Maximal cardiac uptake of ^{99m}Tc-sestamibi was seen after two cycles of DOX, reaching a maximum dose of 12.0%, dropping afterwards. Pairwise comparison returned an adjusted significance of $p = 0.002$ at two cycles. Other organs that displayed a peak dose at two cycles were the kidney and the liver (13.5% and 13.7% respectively). The measured dose in thyroid and intestinal tissue was considerable, peaking at one cycle (7.7%) and four cycles (7.1%) respectively.

Figure 8.3 Biodistribution of radiopharmaceuticals during treatment.

At baseline and after one, two, three or four cycles of DOX administration (corresponding to a dose of 15, 20, 45 or 60 mg/kg).

Figure 8.3 Continued.

^{99m}Tc-Annexin V

The maximal cardiac uptake of ^{99m}Tc-Annexin V was 4.7% after one cycle of treatment (adj. $p = 0.001$), gradually decreasing afterwards to approximately baseline value at four cycles. The highest organ doses were measured in the kidney (19.2%, one cycle), the spleen (5.6% at one cycle) and the liver (8.9% at two cycles). In other organs only small amounts of ^{99m}Tc-Annexin V were measured.

^{99m}Tc-glucuronic acid

The kidney showed the highest uptake of ^{99m}Tc-glucuronic acid, especially at baseline and two cycles (both 9.0%). Cardiac uptake was the lowest compared to the other radiopharmaceuticals and did not show a significant increase with DOX dose. It peaked at four cycles (1.1%).

Figure 8.4 Cardiac distribution of imaging and nonimaging markers at baseline and after DOX administration.

- A. Radiopharmaceutical uptake.
- B. WB markers Bcl-2, Caspase 3 and 8 and p53.
- C. IH markers Caspase 3, TUNEL and HIF-1 α in percentage of positive cells.
- D. Mitochondrial membrane potential markers. Upper and lower ratio (UR and LR).

Figure 8.4 Continued.

^{18}F -FDG

^{18}F -FDG uptake was the highest in the kidney, mainly after three and four cycles (6.4% and 6.3%). Cardiac uptake rose after one week to its peak at three cycles (4.3%, adj. $p = 0.02$). Other organs that displayed high uptake were bone (marrow), liver and intestine (peak dose at four, four and two cycles respectively).

Figure 8.5 Median cardiac uptake of radiopharmaceuticals, stratified according to treatment.

The median uptake differed significantly between DOX-treated and DOX-naïve mice for all radiopharmaceuticals ($p < 0.05$, Mann Whitney U).

Cardiac expression of nonimaging markers

Western Blot analysis of Bcl-2, p53, Caspase 3 and Caspase 8 is depicted in Figure 8.6. Bcl-2 expression reached its minimum at two cycles, although not significant, afterwards increasing to maximum at the cumulative dose of 60 mg/kg. Expression of p53 increased with dose, peaking at four cycles (adj. $p = 0.001$). Caspase 3 expression showed an identical trend by both WB and IH measurement, peaking after the first cycle of DOX (with an adj. $p = 0.004$ and adj. $p = 0.001$, respectively), but decreasing with subsequent cycles to near baseline levels. Caspase 8 expression behaved similarly, with a maximum at one cycle of DOX (adj. $p = 0.007$), afterwards decreasing to baseline level. TUNEL also peaked at one cycle (adj. $p = 0.001$), and dropped with subsequent cycles, although did not return to baseline level. HIF-1 α showed an increase in percentage of positive cells peaking at 0.45 after three cycles (adj. $p = 0.004$), then dropping to 0.27 at maximum DOX dose.

JC-1 expression, depicting $\Delta\psi_m$, showed a decrease of UR during the first two cycles, shortly increasing after three cycles and further decrease after the last cycle. The LR increased initially, but dropped after three cycles and re-increased again during the last cycle.

Table 8.1 Cardiac uptake of investigated markers.

	DOX status	Median uptake in % (SD)					p **
		Baseline	1 cycle	2 cycles	3 cycles	4 cycles	
^{99m} Tc-sestamibi	Treated	3.71 (0.003)	5.06 (0.004)	11.97 (0.030)	5.09 (0.011)	5.02 (0.004)	0.007
	Naïve		3.88 (0.003)	3.79 (0.002)	3.45 (0.002)	3.40 (0.002)	0.143
	Difference		1.18*	8.18*	1.64*	1.62*	
^{99m} Tc-Annexin V	Treated	0.86 (0.002)	4.69 (0.008)	2.65 (0.003)	1.40 (0.001)	1.52 (0.002)	0.002
	Naïve		0.82 (0.001)	0.90 (<0.001)	0.81 (0.002)	0.82 (0.001)	0.925
	Difference		3.87*	1.75*	0.59*	0.70*	
^{99m} Tc-glucuric acid	Treated	0.75 (0.002)	0.80 (0.001)	0.90 (0.001)	1.07 (0.001)	1.10 (0.001)	0.009
	Naïve		0.63 (0.001)	0.70 (0.001)	0.88 (0.001)	0.87 (0.001)	0.082
	Difference		0.17	0.20*	0.19*	0.23*	
¹⁸ F-FDG	Treated	1.51 (0.003)	4.00 (0.002)	4.22 (0.002)	4.28 (0.003)	4.18 (0.004)	0.030
	Naïve		1.48 (0.001)	1.63 (0.002)	1.67 (0.002)	1.67 (0.002)	0.579
	Difference		2.52*	2.59*	2.61*	2.51*	

Table 8.1 Continued.

	DOX status	Median uptake in % (SD)				p **	
		Baseline	1 cycle	2 cycles	3 cycles		4 cycles
		Median expression by WB (SD)					
Bcl-2	Treated	3560 (773)	3305 (437)	2790 (246)	3850 (250)	4750 (189)	0.009
p53		1908 (79)	2395 (132)	2905 (358)	4565 (273)	5185 (189)	0.001
Caspase 3		1575 (234)	3815 (220)	3715 (208)	2255 (172)	2325 (211)	0.002
Caspase 8		1550 (173)	3420 (288)	2630 (215)	1895 (163)	1700 (335)	0.003
		Median expression by IH in % of total cells (SD)					
TUNEL		0.48 (0.14)	5.42 (0.64)	3.56 (0.31)	1.74 (0.20)	1.95 (0.24)	0.001
HIF-1α		0.22 (0.05)	0.36 (0.06)	0.29 (0.02)	0.44 (0.06)	0.27 (0.03)	0.005
Caspase 3		0.18 (0.04)	6.28 (0.58)	4.63 (0.40)	1.66 (0.64)	0.98 (0.23)	0.001

* p <0.05 (Mann-Whitney U). ** p-value by Kruskal-Wallis.
Bold = highest uptake in time; DOX = doxorubicin, SD = standard deviation, WB = Western Blot, IH = immunohistochemistry.

Figure 8.6 Examples of histological and JC-1 analysis in DOX-treated animals.

- A. Western Blot analysis of bcl-2, p53, Caspase 3 and 8. KDa = kilo Dalton.
B. TUNEL and Caspase 3 expression.
C. Δψm. Dot plots of red fluorescence (FL2) versus green fluorescence (FL1) showed high FL2 levels at baseline, indicating intact Δψ. After two cycles of DOX treatment, FL2 decreased remarkably and is progressively restored at cycle three and four

Table 8.2 Correlations of investigated markers stratified to studied mechanism.

	Bcl-2	Caspase 3 (WB)	Caspase 8	p53	TUNEL	HIF-1α	Caspase 3 (IH)
Early apoptosis							
^{99m} Tc-Annexin V	-0.50*	0.91†	0.89†	0.08	0.95†		0.92†
Late apoptosis							
^{99m} Tc-sestamibi	-0.55*				0.38		
^{99m} Tc-glucaric acid	0.28				-0.04		
Perfusion							
^{99m} Tc-sestamibi						0.17	
Glucose metabolism							
¹⁸ F-FDG						0.60†	

Correlations assessed by Spearman's ρ. * $p < 0.05$. † $p < 0.01$.
WB = Western Blot, IH = immunohistochemistry.

Correlations

Next, we calculated Spearman's ρ to determine correlations between imaging and nonimaging markers, stratified to the studied mechanism (table 8.2). Most striking were the correlations between ^{99m}Tc-Annexin V and the Caspases and TUNEL, showing a correlation of 0.89 and higher. Less strong, though significant, were the relations between ^{99m}Tc-Annexin V and Bcl-2 (ρ -0.50), ^{99m}Tc-sestamibi and Bcl-2 (ρ -0.55) and ¹⁸F-FDG and HIF-1α (ρ 0.60). JC-1 measurements were insufficient to calculate exact correlations.

Discussion

This is the first study that evaluated the cardiac response to anthracycline exposure from several mechanistic viewpoints, including early and late apoptosis imaging, perfusion changes and glucose metabolism by a combination of molecular imaging and histological biomarkers.

Early apoptosis imaging – PS expression

^{99m}Tc-Annexin V allows for apoptosis imaging by binding to PS molecules at the outer surface of apoptotic cell membranes.³⁸ The exposure and redistribution of PS marks the start of the apoptotic execution phase and elevated myocardial expression in DOX-treated animals has been associated with the presence of cardiac oxidative stress.²¹⁹ In patients treated with Adriamycin (i.e. another anthracycline) ^{99m}Tc-Annexin V imaging detected cardiomyocyte death at lower dose and earlier stage than echocardiography.²²⁰

In the current study we observed a markedly increased expression of ^{99m}Tc-Annexin V after the first cycle of DOX. This suggests that apoptosis already starts at low levels of anthracycline exposure. Although Bennink et al. have observed a similar early rise of ^{99m}Tc-Annexin V uptake in rat hearts they also demonstrated a dose dependent increase.²²¹ In contrast, we noted a decrease in ^{99m}Tc-Annexin V uptake with subsequent cycles of DOX, confirmed by a firm correlation of ^{99m}Tc-Annexin V with histological apoptosis markers Caspase 3 and 8, indicating true reduction of apoptotic PS expression. Probably only a limited amount of cardiomyocytes is prone to anthracycline-induced apoptosis, which is already reached at low cumulative dose. This supports the observation by Bennink et al. that apoptotic nuclei diminish transmurally in histological evaluation.²²¹

Unlike Bennink et al. our results indicate a strong relationship between TUNEL activity and ^{99m}Tc-Annexin V expression.²²¹ To adequately measure TUNEL activity, cardiac tissue needs to be examined extensively. Probably, as the authors already suggest in their paper, TUNEL activity was missed due to an insufficiently sensitive evaluation method. Indeed, recent research has indicated that DNA degradation belongs to the earliest events of the apoptosis cascade.²¹⁵

Late apoptosis imaging – Membrane disintegration

Cell blebbing and shrinkage and membrane disintegration are events considered to occur late in the apoptotic cascade. This can be assessed by different markers including ^{99m}Tc-glucaric acid (glucarate), which binds to positively charged nuclear histones in the intracellular domain after cardiomyocyte membrane disintegration.²¹⁵ We observed a discrete (though significant) elevation of ^{99m}Tc-glucaric acid compared to controls which minimally but consistently increased with subsequent doses. Cell membrane disintegration represents irreversible cell damage, which is known to be dose-dependent. The similar dose-dependent trend of ^{99m}Tc-glucaric acid suggests that it actually depicts irreversible cardiomyocyte damage, which is of high potential in the diagnosis of chronic AIC. As differences with controls were only mild, the stratification of patients into high and low risk groups could be problematic. Furthermore, we observed a (non-significant) increasing trend among control mice between baseline and cycle four. This could mean that ^{99m}Tc-glucaric acid also depicts physiologic cell turnover.

In addition to cardiomyocyte membrane disintegration, mitochondrial membrane integrity is compromised in AIC. In normal functioning cardiomyocytes ^{99m}Tc -sestamibi is taken up due to a large negative mitochondrial transmembrane potential. When the mitochondrial membrane disintegrates under influence of anthracyclines, the subsequent hyperpolarisation induces an increased uptake of ^{99m}Tc -sestamibi in the mitochondria.²²² In our study DOX-treated animals showed a significant increase in cardiac uptake of ^{99m}Tc -sestamibi until two cycles, followed by a decrease during cycle three and four. This could be explained by the fact that mitochondrial membrane disintegration peaks at medium anthracycline exposure which is supported by our observation of a drop of $\Delta\psi_m$ at two cycles, thus corresponding with ^{99m}Tc -sestamibi uptake. However, ^{99m}Tc -sestamibi kinetics are complex and influenced by other factors like, for example, P-glycoprotein (p-gp) expression. P-gp is a membrane efflux transporter that pumps toxic substances, including DOX, out of cells and ^{99m}Tc -sestamibi is a known substrate for this protein.²²³ Thus, when p-gp is upregulated in response to DOX exposure, which has been observed in murine cardiomyocytes,²²⁴ ^{99m}Tc -sestamibi uptake is decreased accordingly. Unfortunately, we did not evaluate p-gp expression in our model and, to our knowledge, the relationship between p-gp and ^{99m}Tc -sestamibi in cardiomyocytes has not been investigated before.

Perfusion changes

In addition, ^{99m}Tc -sestamibi uptake is influenced by vascular changes that occur following anthracycline administration and resemble the effects of ischemia, illustrated by both reversible and fixed ^{99m}Tc -sestamibi defects.²²² The observed decrease in ^{99m}Tc -sestamibi could therefore also reflect perfusion changes that dominate the abovementioned mechanisms in higher cumulative doses of DOX. To identify whether this occurs it could be helpful to observe the uptake patterns of other ischemia markers like 82-Rubidium, which is incorporated into cardiomyocytes by a different transporter than ^{99m}Tc -sestamibi.²²⁵

Glucose metabolism

In the current study we observed a convincing increase in ^{18}F -FDG directly after commencing anthracycline treatment, which remained at the same level during subsequent doses of DOX. Enhanced ^{18}F -FDG uptake in anthracycline-exposed cardiomyocytes is thought to depend on a neuregulin (NRG) mediated response. NRG is a protein secreted by the endocardium and the endothelium of cardiac vasculature that might protect against AIC and, as an adaptive pattern, enhances glucose metabolism of the heart.²²⁶ Therefore, patients with a higher baseline NRG-expression probably exert a better adaptive response to anthracyclines and those with low cardiac ^{18}F -FDG uptake after anthracycline therapy could be identified as at high risk for AIC early in the process.²²⁷ Such a variable ^{18}F -FDG uptake pattern indeed has been observed in anthracycline-treated lymphoma patients.⁷⁵ Above that ^{18}F -FDG can be used to assess cardiomyocyte viability, since intact cardio-

myocytes utilise glucose for cell functions. Consequently, when cardiomyocytes die, a decreased glucose uptake is observed. The increased cardiac uptake of ^{18}F -FDG throughout the cumulative anthracycline administration in our study suggests a reversible mechanism, since apoptosis apparently does not induce cell death. This is also underlined by the discrete increase of ^{99m}Tc -glucaric acid, which indicates only a limited amount of irreversible cell disintegration.

We observed a strong correlation between ^{18}F -FDG and HIF-1 α , the latter being a hypoxia-driven transcription factor that regulates the expression of a variety of genes in response to a lack of oxygen.²¹⁶ Very recently, Seino et al. have reported a similar relationship in benign abdominal tumours.²¹⁷ They suggest that HIF-1 α expression is enhanced directly after exposure to hypoxia and subsequently activates GLUT-1 and -3, hexokinase, vascular endothelial growth factor and several glycolytic enzymes. Furthermore, since maximal ^{18}F -FDG uptake and HIF-1 α expression were located in areas of increased cellularity but decreased vascularity, ^{18}F -FDG accumulation is not influenced by increased vascular availability or endothelial dysfunction of the radiopharmaceutical, but results from promoting glucose uptake mechanisms as an adaptive mechanism.

Our findings therefore implicate that ^{18}F -FDG can be used to stratify patients into low and high risk groups, but also is able to detect reversibility without being influenced by vascular availability. This is of major importance to guide protective therapy. It provides an interesting opportunity to get clinically exploited, since ^{18}F -FDG nowadays is a widely available and extensively used radiopharmaceutical. Above that, a major advantage of ^{18}F -FDG above other imaging markers is the possibility of a quantitative assessment using the standard uptake value, which provides a comprehensive interpatient and follow up method.

Conclusion

In conclusion, we showed that the cardiac response to anthracyclines at low doses starts with apoptosis depicted by ^{99m}Tc -Annexin V and confirmed by histological apoptosis markers. Late apoptotic changes such as membrane disintegration can be detected by ^{99m}Tc -sestamibi and ^{99m}Tc -glucaric acid. However, ^{99m}Tc -sestamibi uptake is influenced by other mechanisms including p-gp expression and vascular changes that complicate the interpretation of observed uptake patterns. ^{99m}Tc -glucaric acid uptake differed mildly from controls, which implies a problematic differentiation between high and low risk patients. ^{18}F -FDG uptake signifies an early adaptive response to DOX and has the highest potential to serve as a clinically exploitable early marker of reversible cardiotoxicity in the near future.

General discussion

In this thesis, several potential biomarkers for the early detection of anthracycline- and trastuzumab-induced cardiotoxicity (respectively AIC and TIC) have been described and studied. Some are promising, others are not, and this chapter reflects on the implications of these findings.

Choice and validation of parameters

In chapter 2, 3, 4 and 5 we studied strain (rate) imaging, biomarkers, Iodine-123-*meta*-iodobenzylguanidine (¹²³I-*m*IBG) and multigated radionuclide (MUGA) scintigraphy respectively, for their potential value to detect subclinical cardiotoxicity. In addition, the response to doxorubicin exposure of four different radiopharmaceuticals has been evaluated in chapter 8. They all have their pros and cons, especially when logistical disadvantages are added to the balance (Table 9.1). Below we discuss which technique would be the most promising without taking availability issues into account.

Table 9.1 Logistical advantages and disadvantages for the main methods studied in this thesis.

	Noninvasive	Low cost	Patient comfort	Low radiation burden	Availability	Image quality	Safety	Standardisation	Reproducibility
MUGA scintigraphy	+/-	-	-	-	+	+	+	+	+
Strain imaging	+	-	-	+	-	+/-	+	-	+/-
¹²³ I- <i>m</i> IBG scintigraphy	+/-	-	-	-	+	+	+/-	-	+
Nonimaging biomarkers	+/-	+	+	+	+	N/A	+	+	+
^{99m} Tc-Annexin V	+/-	-	-	-	-	+	+	-	+
^{99m} Tc-sestamibi	+/-	-	-	-	+	+	+	+/-	+
^{99m} Tc-glucaric acid	+/-	-	-	-	-	+	+	-	+
¹⁸ F-FDG	+/-	-	-	-	+	+	+	+	+

MUGA

MUGA scintigraphy is an established technique to measure the left ventricle ejection fraction (LVEF) of patients who are treated with anthracyclines or trastuzumab. It is the gold standard, next to echocardiography, and widely used for this purpose.⁵³ Apart from

the LVEF measurement, several other filling parameters can be assessed by MUGA, derived from the time-activity curve (Figure 1.6), including peak filling rate (PFR), peak ejection rate (PER), time to peak filling rate (t-PFR) and time to peak ejection rate (t-PER) and are used to characterise systolic or diastolic function.⁶² These parameters also yield excellent reproducible values.⁵³

For AIC, it is known that diastolic dysfunction (DD) precedes systolic function (SD) due to differences in the susceptibility of different myocardial layers to anthracyclines.⁶⁵ This can be identified by MUGA-derived diastolic parameters.^{64, 164, 165} Although this could lead to the detection of subclinical AIC, this is also a sign of already inflicted damage and would thus be too late in the process to start prophylactic therapy. Furthermore, the mechanism on which TIC is supposedly based, depletion of ATP through HER2 inhibition,²⁷ suggests that all myocytes suffer evenly from trastuzumab toxicity, which will nullify the difference between SD and DD in the case of trastuzumab. This probably explains why we did not find a significant difference.

Strain (rate) imaging

The use of strain imaging in the detection of AIC has been studied more extensively than MUGA, although this is a fairly new technique. When evaluating cardiac function by strain, one can either choose to evaluate global or regional function in the apicobasal plane, which is an advantage over conventional echocardiography assessing global function only.²²⁸ Although this regional assessment can be very useful, in the case of AIC one must concentrate on the global cardiac function, since clinically relevant damage will present evenly in all regions.

Global cardiac strain measurement can be done in different axes (Figure 1.4). In general, the role of global longitudinal strain (GLS) is more important than global radial (or transmural; wall thickening) strain and global circumferential strain (GRS and GCS).⁵⁶ When the heart contracts, the longitudinal axis shortens. Consequently, because the myocardial tissue is considered incompressible and therefore the same volume has to be maintained, this shortening must lead to an increase in transmural volume, resulting in a positive radial strain (Figure 9.1).^{228, 229} This means that GRS is a function of GLS and not an independent parameter. Furthermore, GRS is extremely prone to ROI placement and is influenced heavily by measurement level (i.e. apical, midwall or basal).^{230, 231} Instead of a result of myocardial damage, this is purely due to cardiac geometry. In essence, this also is true for GCS. This renders the reliability of GRS and GCS far less than GLS.

In addition to strain, cardiac function can be assessed by strain rate. Strain rate is the temporal derivative of strain and describes the rate by which deformation occurs. Strain rate shows changes in deformation during each time point in a certain cardiac cycle, while

Figure 9.1 The concept of incompressibility.

- A.** Because the volume (V) of the left ventricle cannot fluctuate (incompressibility), a decrease in the Y-axis needs to be compensated by the same increase in the X-axis, so V stays equal.
- B.** Consequently, a decrease in the Y-axis, will be compensated with an increase in the X-axis, resulting in increased radial strain equal to decreased longitudinal strain.

strain only describes the cumulative deformation during a cycle. Thus, strain rate can detect minor differences in velocity of deformation as the total deformation is not yet affected and is, therefore, thought to be more sensitive than strain.²²⁸ However, the physiology (i.e. velocity) of cardiac deformation is extremely complicated and the value of strain rate in cardiac assessment is definitely not proven yet.²²⁹

Several studies have evaluated the use of GLS in the prediction of AIC. In a study in seventy anthracycline-treated breast cancer patients and fifty healthy controls, Ho observed a significant decrease in GLS, while the LVEF remained within normal limits.⁹⁵ Sawaya and Fallah-Rad separately evaluated the use of GLS in the prediction of AIC in a total of 123 patients. Sawaya concluded that a decrease of 10% of the longitudinal strain or a GLS below -19% immediately after anthracycline treatment predicted subsequent cardiotoxicity.⁸⁴ Fallah-Rad observed a GLS decrease only in patients who developed a decrease in LVEF afterwards or experienced symptoms of congestive heart failure (CHF).¹⁷⁷ Other studies reported comparable results.^{56, 57}

Although this evidence propagates the implementation of GLS as a clinical tool for AIC detection, there are some limitations that might complicate progress into the clinic. One of the most important hurdles is image quality in certain patient groups. Strain imaging uses the typical echocardiographic speckle pattern that is generated by (a part of) the myocardium and follows the movement of this pattern through a cardiac cycle (Figure 9.2). Although regional differences can be identified with this approach, the technique is also more prone to image noise disturbances. Therefore, when echocardiographic image quality is not good, strain imaging is even more difficult to perform than conventional echocardiography. Echocardiographic image quality depends on tissue density as well as the path length that ultrasound waves need to travel. For example, ultrasound waves pass air (not dense) more easily than muscle (dense). Similarly, tissue of one centimetre in thickness is more easily passed than tissue of five centimetres in thickness.

In our cohort of patients, for example, the mean body-mass index was 27.2, which is relatively high (chapter 2). Furthermore, many patients either had an operation or left-sided radiotherapy, which in general increases tissue density. Also, breast implants after reconstruction surgery altered tissue density. This led to insufficient image quality in a significant number of patients, especially for GRS and GCS measurement (28%).

¹²³I-mIBG scintigraphy

The use of ¹²³I-mIBG for the detection of cardiac disease has been studied extensively in the past decades. Some studies concentrated on the prediction of ICD-therapy outcome and stratification of patients at risk.^{47, 49} However, other studies have assessed the use of the H/M ratio in cardiac failure. As early as 2001, a study in 79 patients showed that cardiac washout (WO) was a predictor of chronic heart failure outcome (cut off 27%).⁵² In a systematic review by Verberne et al. in a total of 1175 patients, the estimated hazard ratio of an increased washout was 1.72 for cardiac death. Although the calculation of the H/M ratio was diverse, the pooled hazard ratio of the three qualitative best studies, with fairly comparable H/M ratio calculation, turned out to be 1.82 for cardiac death and 1.98 for cardiac events.⁵¹ The ADMIRE-HF trial again demonstrated the prognostic power of ¹²³I-mIBG parameters.⁵⁰

On the other hand, the role of ¹²³I-mIBG scintigraphy in the detection of AIC is not studied well. Despite the promising results of some in vitro studies, that have shown a decrease of cardiac ¹²³I-mIBG uptake before morphologic and LVEF changes are observed, no clinical studies other than a case series have been performed on this subject.^{38, 232} Chapter 4 of this thesis is the first description of ¹²³I-mIBG indices in a homogenous group of breast cancer survivors one year after therapy. It shows that there are correlations between the H/M ratio and, for example, GLS and GRS.

Figure 9.2 Basics of speckle tracking.

A region of interest (green) is drawn in the left ventricle. This region has a particular signature of acoustic reflections ('speckles'). This signature can be followed during the cardiac cycle and converted to a graph (not shown).

When looking at the pathophysiologic mechanism of AIC, different phases in the process can be identified. Activation of the sympathetic nervous system, which is the base for changes in the H/M ratio and WO, occurs early in the process, even before structural changes are observed. This suggests that it would be a perfect marker directing prophylactic anti-cardiotoxic therapy. However, in chapter 4 no general decrease in H/M ratio was found. This could be due to the incidence of AIC in our relatively small group, the time point chosen for evaluation or the lack of sensitivity of the method. Despite this, ¹²³I-mIBG scintigraphy should not be dismissed as a method for early detection of AIC, but should be prospectively evaluated in a larger cohort.

^{99m}Tc-Annexin V

The apoptosis imaging agent ^{99m}Tc Annexin V has been studied in a large number of different tumour types and suggested to be of value in the assessment of chemotherapy

and radiotherapy response in several patient series.²¹⁵ It has shown to indicate the early apoptotic changes in cancer cells in a safe and reproducible way.²³³ Although some attempts have been made to study the cardiac response to anthracyclines, and the potential of the radiopharmaceutical to identify cell damage before echocardiography has been recognised,²²⁰ ^{99m}Tc-Annexin has not yet found its way to human cardiotoxicity imaging.

However, in chapter 8 a strong relationship was observed between ^{99m}Tc-Annexin and several histological apoptosis markers, occurring at low cumulative dose of anthracycline administration, which underlines the potential of this radiopharmaceutical in the early detection of AIC. Perhaps, an H/M ratio analogue to ¹²³I-*m*IBG scintigraphy could be implemented to assess cardiac apoptosis in a semi-quantitative manner.

¹⁸F-FDG

Although ¹⁸F-FDG has been used for decades to image oncologic and inflammatory diseases, and, at the same time, assess cardiac viability, it has not been until recently that ¹⁸F-FDG became apparent as a potential molecular marker for early AIC. In theory, the radiopharmaceutical is perfect for it: experience is extensive, availability is good, image quality is superior, costs are constantly decreasing and, most important, it can detect AIC early in the process due to a neuregulin-mediated adaptive response while it excludes necrotic tissue since these cells do no longer utilize glucose. Furthermore, a comprehensive tool for quantitative assessment of tissue has already been developed and can be used immediately to serve as a solid and reproducible parameter.

The exact mechanism by which glucose metabolism is influenced in AIC (or TIC) has not been studied. This is of course of great importance, since it will potentially guide prophylactic cardiac therapy in the near future either by stratifying patients into low and high risk groups, or by separating reversible from irreversible cardiotoxicity. This should be assessed in a clinical setting, investigating the effects of both anthracyclines and trastuzumab on cardiac glucose metabolism.

^{99m}Tc-sestamibi and ^{99m}Tc-glucuric acid

Similar to ¹⁸F-FDG, ^{99m}Tc-sestamibi has been used for a long time in nuclear medicine to detect perfusion changes of the heart and image certain tumours. Since it was recognised that ^{99m}Tc-sestamibi also represents mitochondrial membrane changes, only few (pre) clinical studies have tried to implement the radiopharmaceutical in the detection of drug-induced cardiotoxicity.²²² As described in chapter 8, ^{99m}Tc-sestamibi uptake is complex and influenced by perfusion changes, mitochondrial membrane integrity and P-glycoprotein (P-gp) expression. This hampers the interpretation of ^{99m}Tc-sestamibi uptake patterns in such a way that for now ^{99m}Tc-sestamibi is not suitable as an AIC marker.

However, when the parameters that influence cardiac uptake could be identified, ^{99m}Tc-sestamibi could be of value, since experience with it is extensive.

In contrast, ^{99m}Tc-glucuric acid is a relatively new radiopharmaceutical that is experimentally used for the detection of irreversibly injured cardiomyocytes, for example after myocardial infarction.⁷⁴ When myocytes necrotise, ^{99m}Tc-glucuric acid enters the cell and uptake is enhanced. Potentially, this marks an important change in the process of AIC, allowing to separate early (reversible) and late (chronic) cardiotoxicity. However, information on the exact kinetics of ^{99m}Tc-glucuric acid is not yet available and should be evaluated in patients before a substantial hypothesis can be drafted on the use of this radiopharmaceutical for AIC imaging.

Nonimaging biomarkers

As discussed in chapter 3, the use of troponin I (TnI) – especially its high-sensitive variant hs-troponin I – has gained considerable support as a clinical biomarker of early AIC. Recent studies have only strengthened this belief. Ky et al. report a negative predictive value of 99% for normal hs-TnI levels directly and one month after anthracycline administration and Cardinale et al. suggest that the extent of LVEF decrease can be predicted by TnI.^{155, 234} Systematic reviews on this topic conclude that hs-TnI has great potential in the early detection of AIC.^{234, 235} Indeed, following the National Cancer Institute, the European guideline on cardiotoxicity advocates the use of TnI as a clinical marker of AIC.²³⁶

Another interesting biomarker is NT-proBNP, which returned abnormal results in 18% of patients in chapter 3, correlating with LVEF changes. In literature, the evidence on NT-proBNP as a biomarker for adult AIC is growing.^{112, 122, 123, 237, 238} In general, studies favour the role of NT-proBNP as a predictor of AIC, even when no echocardiographic or clinical changes have occurred.^{112, 239} However, most studies consist of heterogenic patient groups and the biomarker lacks both specificity and sensitivity when compared to (hs)TnI.

For the other biomarkers that we evaluated in chapter 3, the evidence is scarce. TNF-α and IL-6, both pro-inflammatory cytokines and markers of tissue damage, have been found to increase in response to myocyte apoptosis.^{240, 241} However, to date no evidence has emerged that neither TNF-α nor IL-6 can play a predictive role in the early diagnosis of AIC.

ST2 is a new biomarker, depicting inflammation and fibrosis, and belongs to the family of interleukin-I receptors. A recent review shows the tremendous amount of research on this biomarker that has been initiated in a vast diversity of diseases, including acute and chronic HF, generating conflicting results.²⁴² At the time of enrolment of patients in our cohort, no studies on the use of ST2 (or sFlt-1) as a biomarker for AIC had been described. The first study that evaluated ST2 in this field did not observe a change in the level of ST2

during the one year follow up, although baseline levels were considerably high.⁸⁴ The findings in our cohort were comparable: no increased levels were observed.

Soluble Fms-like tyrosine kinase 1 (sFlt-1) is another emerging biomarker, which depicts vascular remodelling. The first study that describes this marker in the process of AIC returned increased levels during follow-up, but lacked significant predictive power.¹⁵⁵ Our cohort did not show any increased levels of sFlt-1 one year after anthracycline treatment.

Galectin-3, a protein expressed by macrophages and a marker of fibrosis, predicts cardiac events in acute HF.¹⁵⁶ The same group of investigators that studied sFlt-1 in AIC, evaluated galectin-3 and concluded that there was no association between galectin-3 levels within three months of anthracycline administration and the development of AIC. In our cohort, we observed abnormal levels of galectin-3 in four patients (7.3%). The value of these results is hard to interpret, as follow-up levels of this biomarker have not been investigated before. For now, discarding Galectin-3 as invaluable for early AIC seems premature.

Standardisation

As indicated above as well as the different chapters of this thesis, standardisation issues are a very important limiting factor in the implementation of new biomarkers.

For imaging biomarkers, the main challenge for standardisation is the choice of the parameter and the method by which it is obtained. Of the studied methods, experience with MUGA scintigraphy is extensive and standardisation issues regarding the acquisition and processing have been settled in time. Also, the choice of parameters is quite straightforward, making standardisation issues in MUGA scintigraphy negligible.

The same holds true for ¹⁸F-FDG, since a generally accepted parameter for the evaluation of metabolic activity (i.e. standardised uptake value; SUV) has been developed in recent decades. This quantitative marker represents the relative intensity of a certain region of interest (ROI), for example the heart, compared to the mean body intensity and is influenced by body weight and time after injection.²⁴³ Furthermore, European imaging protocols have been developed and adapted to aid in the harmonisation of ¹⁸F-FDG imaging.²⁴⁴ Despite these efforts, SUVs can be influenced by different factors such as image noise and resolution and ROI selection and ¹⁸F-FDG uptake can vary over 30% on a day-to-day basis.²⁴⁵ However, in general the SUV is accepted as a solid, reproducible and easy-to-use parameter that can be used to quantitatively assess cardiac uptake, which is a major advantage over other imaging markers.

For strain imaging the parameter of choice is GLS, which is widely accepted throughout literature. However, the use of different software packages poses a potential problem.²²⁹ In general, EchoPAC is the commercial software package that is used most often, but in literature authors tend to use self-designed software packages, introducing 'inter-vendor' reproducibility errors. This problem can be solved by making sure that the underlying algorithms are identical, especially with regards to automated segment tracking.²²⁸

Although there have been efforts to standardise acquisition protocols for ¹²³I-mIBG scintigraphy, for example in the guideline of Flotats,⁴⁶ there are still several remaining issues that need attention.⁴⁵ Furthermore, the use of many different parameters that can be derived from ¹²³I-mIBG scintigraphy needs structure. In chapters 4, 6 and 7 different standardisation issues have been addressed. Three important ones are the time-to-acquisition (i.e. fifteen minutes or four hours post injection), acquisition method (i.e. on planar or SPECT images; posterior, geometric mean or anterior view) and cardiac ROI placement (Figure 9.3). Another important issue, the choice of collimator, was not addressed in this thesis, but is extensively reviewed by other authors.^{45,46}

Chapter 4 showed that the delayed planar anterior WH H/M ratio was the most robust. This is in line with most literature, in which the delayed H/M ratio is used, but the standardisation proposal by Flotats states that 'more studies are needed to establish the differences between these parameters'.⁴⁶ Other results of this thesis (chapter 4) include the observation that obtaining a posterior view for geometric mean calculation did not provide any added value, nor did the placement of ROIs other than the WH method. Furthermore, SPECT-indices, although probably offering a more accurate depiction of the H/M ratio, is not recommended because of differences in acquisition standardisation. This eliminates some issues and brings the implementation of ¹²³I-mIBG scintigraphic parameters as a marker for subclinical AIC somewhat closer.

In general, nonimaging biomarkers do not suffer from these standardisation limitations. However, there are some problems that apply for both imaging and nonimaging biomarkers. The most important ones are the number and timing of the measurements and the threshold that should be used to detect cardiotoxicity.^{234, 235, 246} This is recognised by the European guideline on cardiotoxicity, which advises the measurement of TnI before and after every cycle of anthracyclines, without giving a threshold value.²³⁶ That the timing of the measurement is essential, is underlined by the fact that our study cohort did not display abnormal TnI levels one year after treatment. Other groups also observed a descending trend in hs-TnI levels in time or a return to normal levels.^{84, 122} This marks the importance of a standardised approach in the assessment of AIC by TnI, but is also valid for other biomarkers, although GLS seems to fluctuate less in the course of follow up (chapter 2).^{84, 99, 247}

Figure 9.3 Cardiac ROI placement in ^{123}I -mIBG scintigraphy.

- A. 'Whole heart' ROI, including the myocardial cavum.
- B. Left ventricle ROI.
- C. Small left ventricle ROI.

The final issue on standardisation that needs to be mentioned is the heterogeneity of patient groups. Studies on cardiotoxicity are often performed in childhood cancer survivors or heterogenic patient groups, since cardiotoxicity is a rare complication that can occur up to decades after treatment. Although understandable, such heterogeneity interferes with the results and hampers the translation of the biomarker to clinical protocols. This emphasises the need for the implementation of cardiotoxicity biomarkers in drug trials, which will be discussed in the next paragraph.

Implementation of standard biomarkers in trials with cardiotoxic drugs

Anthracyclines have been used for decades to treat breast cancer and the first reports of AIC date back to the early 1970's. The limited life expectancy that those patients faced, when treated with anthracyclines, is probably the main reason why no prospective studies on late AIC have been performed in (homogenous groups of) breast cancer patients. Since in recent years the life expectancy has improved by patient-tailored treatment, identifying strategies to reduce the complications of anticancer therapy have gained interest. Nonetheless, we are still struggling with AIC and TIC, and optimal methods to detect this problem have not been developed yet.

Research on new therapeutics is ever ongoing and undoubtedly new drugs will be introduced in the coming years, thereby further improving the survival of breast cancer patients. However, if we want to prevent cancer survivors suffering from (often unknown) toxic side effects, it is obligatory to include high-potential biomarkers into phase 2 and 3 studies of these new therapeutics. Not only will this improve the detection of potentially harmful side effects, but also will this induce the possibility to compare the potential methods prospectively in terms of accuracy and predictive value, as well as costs, logistic challenges and standardisation issues mentioned above. As no additional studies need to be performed, it will save time and money. Above that, patients will benefit from safer introduction of new therapeutics.

With regard to cardiotoxicity, the most important research topic is probably the development of a panel of biomarkers that is able to stratify patients into three risk categories: (1) no cardiotoxicity (low risk), (2) transient cardiotoxicity (medium risk) and (3) chronic cardiotoxicity (high risk). Group 2 would require close monitoring and group 3 prophylactic therapy or change to a different therapeutic regimen. Ky and Sawaya initiated research on a combined approach of different biomarkers (hs-TnI and myeloperoxidase and GLS and hs-TnI I, respectively) and have shown that this has the potential to predict drug-induced cardiotoxicity in breast cancer patients.^{84, 155} However, a stratified approach would make assessment of patients more clear and provide a tailored treatment, improving the quality of life of cancer survivors.

As has been shown in chapter 8 radiopharmaceuticals could be of great value in the stratification of patients at risk and pinpoint certain phases in the cardiotoxic process, but need additional research to enable correct interpretation of uptake patterns. Special attention should be paid to the imaging of apoptosis and glucose metabolism with $^{99\text{m}}\text{Tc}$ -Annexin V and ^{18}F -FDG early after anthracycline administration and necrosis imaging with $^{99\text{m}}\text{Tc}$ -glucaric acid later on. Unfortunately, preparation kits for the radiolabelling of $^{99\text{m}}\text{Tc}$ -Annexin V and $^{99\text{m}}\text{Tc}$ -glucarate acid are not commercially available, implicating that these tracers are only to be used in specialised research institutes.

Although some research has been done to define the most effective prophylaxis of treatment-induced cardiotoxicity, current treatment protocols have been deduced from general CHF guidelines and indicate a role for antihypertensive medication like diuretics, angiotensine converting enzyme inhibitors (ACEi) and beta blockers.²⁴⁸ With respect to AIC, several studies have shown the potential value of enalapril (when possible in combination with carvedilol), dexrazoxane and statins.^{15, 249-251} However, these studies suffer from low patient numbers, methodological flaws, and heterogenic patient groups. Furthermore, anti-cardiotoxic treatment should not negatively affect antitumor efficacy, which has been suggested by some authors.¹⁵ Therefore, this is also a topic that requires an adequately powered, prospective trial in a homogenous group of patients.

Conclusions

In conclusion, of the clinically studied biomarkers MUGA scintigraphy is the least promising in the detection of subclinical cardiotoxicity, irrespective of whether it is caused by anthracyclines or monoclonal antibodies. ^{123}I -mIBG scintigraphy still has several standardisation issues that hamper its implementation as a clinical biomarker, but the delayed H/M ratio, together with WO, are potentially useful and need to be evaluated in a large prospective study that includes baseline measurements. Current evidence on NT-proBNP mainly discards its role as a potential biomarker due to a lack of sensitivity and specificity. For $^{99\text{m}}\text{Tc}$ -sestamibi and $^{99\text{m}}\text{Tc}$ -glucaric acid, the cardiac uptake mechanisms should be addressed before a clinical study can be performed.

The most promising biomarkers studied in this thesis are ^{18}F -FDG, $^{99\text{m}}\text{Tc}$ -Annexin V and GLS. Although TnI did not return abnormal results in our cohort, evidence yielded by other study groups is convincing. Albeit minor standardisation issues need to be addressed, GLS and TnI should be implemented in the clinical work-up on AIC (and TIC).

Finally, phase 2 and 3 studies on new anticancer therapeutics need to implement (combinations of) biomarkers (i.e. GLS, TnI in combination with either ^{123}I -mIBG scintigraphy, ^{18}F -FDG and $^{99\text{m}}\text{Tc}$ -Annexin V) in their study protocols in order to assess potential cardiotoxicity early in the process, at the same time determining the usefulness of separate and combined biomarkers. The lack of commercial availability of GLS and $^{99\text{m}}\text{Tc}$ -Annexin V potentially restrict their use in these trials.

Furthermore, prospective evidence should also be acquired to determine the most effective prophylactic treatment.

10

English and Dutch summary

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Unpublished

10.1 English summary

In this thesis, the role of several imaging and nonimaging markers for the detection of anthracycline-induced and trastuzumab-induced cardiotoxicity (respectively AIC and TIC) is evaluated. Especially, the pathophysiology of these processes and the interrelationship of the various markers are emphasised.

Cardiotoxicity can be divided in acute, early-onset chronic and late-onset chronic cardiotoxicity. Acute cardiotoxicity is usually reversible and self-limiting, while the chronic variant persists after discontinuation of therapy and can potentially lead to congestive heart failure, arrhythmia and even death, up to twenty years after treatment. Since the survival of breast cancer patients keeps improving, the role of chronic cardiotoxicity as a long-term side effect provides new challenges in the management of patients. Furthermore, the increasing frequency of trastuzumab (and analogues like pertuzumab) as an adjuvant agent in human epidermal receptor 2 (HER2)-positive breast cancer could further increase the incidence of serious cardiac side effects. The current aim of cardio-oncologists is therefore to identify patients at risk of developing these problems in a minimally invasive way. To date, cardiac function is assessed by conventional echocardiography or multigated radionuclide angiography (MUGA) and expressed as left ventricle ejection fraction (LVEF).

In the first part of this thesis, the findings of the TOXTAC study are presented. In this study, a cohort of anthracycline-treated breast cancer patients was assessed with different methods that probably are able to detect AIC. One of these, 2D strain imaging, is a new echocardiographic method to image the relative deformation of the cardiac wall. In 18% of our patients, global longitudinal strain (GLS) was decreased one year after treatment, while conventional echocardiographic parameters (including LVEF) remained normal. Furthermore, strain rate measurements, which depict the rate of wall deformation, showed a significant decrease compared to reference groups. Of the evaluated biomarkers, only N-terminal pro brain natriuretic peptide (NT-proBNP) showed promising results, returning an abnormal value in 18% of patients and significantly correlating with LVEF. Troponin I was not elevated, nor were other biomarkers like tumour necrosis factor α (TNF- α), soluble Fms-like tyrosine kinase 1 (sFlt-1), ST2 and galectin-3. The molecular imaging biomarker Iodine-123-*meta*-iodobenzylguanidine (^{123}I -mIBG) was assessed, to depict the sympathetic response of the heart. Of the available parameters derived from this method, the delayed whole heart (WH) heart-to-mediastinum (H/M) ratio proved to be the most robust. Global radial strain was the only parameter of the other methods that predicted the delayed WH H/M ratio by multivariate analysis.

Although trastuzumab in general induces reversible cardiotoxicity, it could become chronic, affecting the quality of life and aggravating AIC. The occurrence of TIC is therefore monitored by LVEF measurements through MUGA, indicating systolic dysfunction (SD). However, diastolic dysfunction (DD), which can also be evaluated by MUGA, might occur before SD, enabling detection of TIC before functional impairment takes place. In our study we could not find a significant difference in the time-to-occurrence of DD compared to SD. On the other hand, in 27% of patients a decrease in diastolic function without SD was seen, while only 16% of patients showed a decrease in systolic function without DD. In a small subgroup of patients with advanced (metastasised) breast cancer, 85% of patients developed DD, while 54% developed SD, but time-to-occurrence did not differ. The time-to-occurrence did differ in the subgroup of anthracycline-naïve patients. In conclusion, MUGA-derived DD seems unsuitable for the early detection of TIC.

Many factors can affect the ^{123}I -mIBG scintigraphic H/M ratio. Two of them, the presence of catecholamines in the circulation and the cardiac region of interest (ROI), were examined in this thesis. We showed that circulating catecholamines, which are secreted by several hormonal active tumours, affect the calculation of the H/M ratio in such a way, that reliable assessment of the cardiac function by ^{123}I -mIBG scintigraphy is not possible. This means that for children with neuroblastoma, often receiving high doses of anthracyclines, another method should be developed to assess their cardiotoxicity risk. The second factor, ROI placement, was also studied in this patient group. The ROI is the region of the heart (and the mediastinum) that is included in the H/M ratio calculation. The mediastinal ROI is generally drawn in the same way throughout literature, but for the heart ROI different approaches are used: the whole heart region, the left ventricular wall region and the small left ventricular wall region. For our patient group we concluded that the WH method should be used, since it provides the best interobserver agreement. However, in a patient group that contains patients with a dilated cardiomyopathy, this method could underestimate the H/M ratio since it includes a relatively large part of cardiac blood pool. For these patients, the left ventricular wall ROI is probably more reliable.

In the last chapter of this thesis we evaluated cardiac metabolism during anthracycline administration. In this preclinical study, mice were exposed to one, two, three or four cycles of doxorubicin, while cardiac molecular mechanisms were monitored by $^{99\text{m}}\text{Tc}$ -Annexin V, $^{99\text{m}}\text{Tc}$ -sestamibi, $^{99\text{m}}\text{Tc}$ -glucaric acid and ^{18}F -FDG. Also, after each cycle mice were sacrificed to study the cardiac expression of several nonimaging markers including Bcl-2, Caspase 3 and 8, TUNEL, HIF-1 α , p53 and JC-1. We observed a significant increase of cardiac uptake of all radiopharmaceuticals as compared to control mice. $^{99\text{m}}\text{Tc}$ -Annexin V uptake increased at low cumulative anthracycline dose and correlated strongly to histological apoptosis markers, suggesting to allow apoptosis imaging early in the process of cardiotoxicity. ^{18}F -FDG also increased at low cumulative dose and remained

high during subsequent doses, implicating a fast glucose-mediated adaptive response, but also a high potential for reversibility, which could be used for patient risk stratification.

10.2 Nederlandse samenvatting voor niet-ingewijden

Dit proefschrift beschrijft het onderzoek naar de waarde van diverse methodes voor het zo vroeg mogelijk herkennen van achteruitgang van de hartfunctie na anti-kanker behandeling met anthracyclines en/of trastuzumab. Daarbij wordt in het bijzonder aandacht besteed aan het ontstaan van deze hartklachten op moleculair niveau en de relatie tussen de diverse parameters die worden gebruikt om dit in kaart te brengen.

Aantasting van het hart (cardiotoxiciteit genoemd) kan op drie momenten na start van de anti-kanker behandeling naar voren komen: acuut (direct na toediening), vroegtijdig chronisch (binnen een jaar na toediening) en laat chronisch (meer dan een jaar na toediening). Acute cardiotoxiciteit is meestal niet blijvend van aard, terwijl chronische cardiotoxiciteit blijvende problemen veroorzaakt, bestaand uit onder andere hartfalen, ritmestoornissen en zelfs overlijden. De chronische variant kan nog tot twintig jaar na chemotherapie optreden en het vóórkomen hiervan wordt geschat op maximaal 5% van de behandelde patiënten.

Chemotherapie met antracyclines is nog steeds een belangrijk onderdeel van de behandeling van patiënten met borstkanker. In de afgelopen decennia is de behandeling dusdanig verbeterd dat patiënten langer leven en dus meer kans maken om de nadelige lange-termijneffecten van anthracyclines te ervaren. Het is daarom van belang patiënten die een verhoogd risico op deze bijwerkingen hebben op tijd te identificeren, zodat er gericht actie kan worden ondernomen (bijvoorbeeld door bepaalde medicijnen voor te schrijven).

Een tweede verandering in de behandeling van patiënten met borstkanker is het toegenomen gebruik van trastuzumab (merknaam: Herceptin) bij patiënten bij wie de tumor bepaalde kenmerken laat zien. Hoewel trastuzumab (en soortgelijke medicijnen) krachtige middelen zijn die de behandeling verder verbeteren, hebben deze eveneens een nadelige uitwerking op het hart. Een combinatie van anthracyclines en trastuzumab kan de kans op cardiale problemen vergroten. Dit is nog meer reden om risicopatiënten vroegtijdig te identificeren. Tot nu toe worden deze patiënten gevolgd middels echocardiografie of nucleaire ejection fractie bepaling, waarbij de pompfunctie van de linkerkamer (LVEF) wordt bepaald.

In het eerste gedeelte van dit proefschrift worden de bevindingen uit de TOXTAC-studie gepresenteerd. In deze studie werden patiënten die zijn behandeld met anthracyclines, na één jaar teruggezien en onderzocht middels 2D strain imaging, jodium-123-*meta*-iodo-benzylguanidine (¹²³I-*m*IBG) scintigrafie en verschillende bloedbepalingen.

2D strain imaging is een nieuwe echocardiografische methode om de relatieve vervorming van de hartwand te bepalen. In 18% van onze patiënten was één van de strain parameters (global longitudinal strain, GLS) afwijkend, terwijl de 'gebruikelijke' echoparameters (onder andere de LVEF) normaal bleven. Daarnaast toonden de strain rate parameters, die het tempo van de vervorming aangeven, een significante afname ten opzichte van een referentiegroep. Van de geanalyseerde bloedbepalingen liet alleen N-terminal pro brain natriuretic peptide (NT-proBNP) een abnormale waarde zien in 18% van de patiënten en was er sprake van een relatie tussen NT-proBNP en LVEF (maar niet tussen NT-proBNP en GLS). Een andere veelbelovende marker, Troponine-I, liet geen afwijkende waarden zien, evenals de overige bloedbepalingen.

Bij ^{123}I -mIBG scintigrafie wordt gebruik gemaakt van een radioactief middel dat vergelijkbaar is met de lichaamseigen stof noradrenaline. Noradrenaline functioneert onder andere als een signaalstof tussen zenuwuiteinden in het autonome zenuwstelsel (ook wel sympathisch zenuwstelsel). Het autonome zenuwstelsel heeft ook invloed op de functie van het hart en reageert op prikkels van buitenaf, zoals toediening van cardiotoxische medicijnen. Omdat ^{123}I -mIBG, in tegenstelling tot noradrenaline, niet wordt afgebroken nadat het is opgenomen door hartcellen, kan er een beeld gevormd worden van de sympathische reactie van het hart op cardiotoxische middelen. Van de beschikbare parameters binnen de ^{123}I -mIBG scintigrafie scan was de 'late whole heart heart/mediastinum ratio' (delayed WH H/M ratio) het meest robuust. Van de overige methoden die werden onderzocht, voorspelde alleen global radial strain de uitkomst van de late WH H/M ratio.

Het effect van trastuzumab op het hart wordt momenteel in kaart gebracht door de nucleaire ejectefractiebepaling, ook wel multigated radionuclide angiografie (MUGA). Hoewel trastuzumab in de regel slechts omkeerbare cardiotoxiciteit veroorzaakt, kan dit chronisch worden als het niet tijdig wordt onderkend. Het opsporen van cardiotoxiciteit door trastuzumab met MUGA kan zowel door analyse van de systolische functie ('pompfunctie'), als door analyse van de diastolische functie ('ontspanfunctie') van het hart. Het is bekend dat bij gebruik van anthracyclines de ontspanfunctie afneemt voordat de pompfunctie afneemt. Hoewel een verminderde ontspanfunctie in onze populatie van trastuzumab gebruikers vaker voorkwam dan een verminderde pompfunctie, vonden wij geen relevant verschil in de tijd tot optreden van één van beiden, behalve in een kleine subgroep van patiënten die nooit met anthracyclines waren behandeld. Concluderend lijkt, voor de opsporing van cardiotoxiciteit door trastuzumab, het bepalen van de ontspanfunctie door MUGA geen relevante toevoeging te zijn op het bepalen van de pompfunctie.

Vervolgens werd gekeken naar twee factoren die de H/M ratio op ^{123}I -mIBG scintigrafie beïnvloeden: de aanwezigheid van catecholamines in de bloedbaan en de cardiale regio of interest (ROI). We toonden aan dat in de bloedbaan aanwezige catecholamines, die worden uitgescheiden door verschillende hormonaal actieve tumoren, de berekening van de H/M ratio dusdanig verstoren dat de hartfunctie niet betrouwbaar beoordeeld kan worden met ^{123}I -mIBG scintigrafie. Dit betekent dat voor kinderen die lijden aan neuroblastoom (een hormonaal actieve tumor) en hiervoor vaak hoge doses anthracyclines krijgen toegediend, een andere methode moet worden gebruikt om hun risico op cardiotoxiciteit vast te stellen. De tweede factor, plaatsing van de regio van interesse (ROI), werd ook onderzocht in deze patiëntgroep. De ROI is het gedeelte van het hart (en het mediastinum) dat wordt gebruikt voor de berekening van de H/M ratio. Over de manier waarop de ROI over het hart wordt geplaatst is de medische literatuur niet eenduidig: men gebruikt een ROI die over het gehele hart wordt gelegd (WH), over de gehele linkerkamerwand of slechts over een gedeelte van die linkerkamerwand. Voor onze patiëntgroep, kinderen dus, kan het beste de WH-methode worden gebruikt, omdat deze de kleinste foutmarge geeft bij bepaling door verschillende onderzoekers. Echter, in een patiëntgroep die ook patiënten met een vergroot hart bevat, kan deze manier van ROI-bepaling de H/M ratio onderschatten. Voor deze patiënten is de linkerkamerwand ROI wellicht betrouwbaarder.

Tot slot werd de reactie van het hart op anthracyclines geëvalueerd in een proefdierstudie. In deze studie werden muizen blootgesteld aan één, twee, drie of vier kuren doxorubicine (een anthracycline), waarbij het de reactie van het hart werd bepaald met een viertal radioactieve middelen: $^{99\text{m}}\text{Tc}$ -Annexin V, $^{99\text{m}}\text{Tc}$ -sestamibi, $^{99\text{m}}\text{Tc}$ -glucaric acid en ^{18}F -FDG. Daarnaast werd na elke kuur bij aantal muizen de expressie van diverse weefselbepalingen (Bcl-2, Capsase 3 en 8, TUNEL, HIF-1 α , p53 and JC-1) onderzocht. Er werd, vergeleken met controlemuizen, een verhoogde stapeling van de radioactieve middelen in het hart gevonden. De opname van $^{99\text{m}}\text{Tc}$ -Annexin V nam reeds toe bij een lage dosis anthracyclines en toonde een sterke samenhang met de weefselbepalingen die geprogrammeerde celdood (apoptose) identificeren. Dit suggereert dat $^{99\text{m}}\text{Tc}$ -Annexin V kan worden gebruikt om apoptose vroeg in het proces van cardiotoxiciteit zichtbaar te maken. ^{18}F -FDG opname liet ook een vroege stijging zien en bleef aanzienlijk verhoogd gedurende de daaropvolgende kuren doxorubicine. Dit impliceert een vroege (glucose-gereguleerde) aanpassing van de hartcellen, maar eveneens dat deze niet direct afsterven. Dit kan van belang zijn voor risicobepalingen binnen patiëntgroepen.

Appendices

List of abbreviations

References

List of publications

Curriculum vitae in English and Dutch

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List of abbreviations

$\Delta\psi_m$	- Mitochondrial membrane potential
(hs)-TnI	- High-sensitive troponin I
^{123}I -mIBG	- Iodine-123- <i>meta</i> -iodobenzylguanidine
^{18}F -FDG	- Fluorine-18-fluorodeoxyglucose
2D	- Two-dimensional
$^{99\text{m}}\text{Tc}$	- Technetium-99- <i>metastable</i>
AC	- Adenyl cyclase
ACE(i)	- Angiotensin-converting-enzyme (inhibitor)
AE	- Adverse events
AIC	- Anthracycline-induced cardiotoxicity
AT-II	- Angiotensine II
ATP	- Adenosine triphosphate
AVC	- Aortic valve closure
BMI	- Body mass index
BSA	- Body surface area
CHF	- Congestive heart failure
CI	- Confidence interval
CMF	- Cyclophosphamide, methotrexate, fluorouracil
CMR	- Cardiac magnetic resonance imaging
CRP	- C-reactive protein
CV	- Coefficient of variation
DD	- Diastolic dysfunction
DNA	- Deoxyribonucleic acid
DOX	- Doxorubicin
E/A ratio	- Early and late diastolic transmitral peak flow velocity ratio
E/e' ratio	- Early diastolic peak flow to annular flow velocity ratio
ECG	- Electrocardiography
ED(V)	- End diastole (volume)
EDTA	- Ethylenediaminetetraacetic acid
ELISA	- Enzyme-linked immunosorbent assay
ES(V)	- End systole (volume)
FEC	- Fluorouracil, epirubicin, cyclophosphamide
FITC	- Flow cytometry
GBq	- Gigabecquerel
GCS	- Global circumferential strain
GCSR	- Global circumferential strain rate
Geo	- Geometric mean
GLS	- Global longitudinal strain
GLSR	- Global longitudinal strain rate
GLUT	- Glucose transporter
GRS	- Global radial strain
GRSR	- Global radial strain rate

Gy	- Gray
H/M ratio	- Heart-to-mediastinum ratio
HbA1c	- Hemoglobin A1c
HDL	- High-density lipoprotein
HER2	- Human epidermal receptor 2
HF	- Heart failure
HIF-1 α	- Hypoxia-inducible factor 1 α
HVA	- Homovanillic acid
ICC	- Intraclass correlation coefficient
ICD	- Implantable cardiac defibrillator
ID/g	- Injected dose per gram
IH	- Immunohistochemistry
IL	- Interleukine
i.p.	- Intraperitoneal
IRW	- Inveon research workplace
i.v.	- Intravenous
IVSd	- Interventricular septal wall thickness at end diastole
keV	- Kilo electro Volt
LAEDV	- Left atrial volume at end diastole
LAO	- Left anterior oblique
LCC	- Lin's concordance correlation coefficient
LDL	- Low-density lipoprotein
LR	- Lower border
LV(D)	- Left ventricle (dysfunction)
LVAW	- Left ventricle anterior wall
LVEDD	- Left ventricular end-diastolic diameter
LVEDV	- Left ventricular volume at end diastole
LVEF	- Left ventricle ejection fraction
LVESD	- Left ventricular end-systolic diameter
LVESV	- Left ventricular volume at end systole
LVIDd	- Internal dimension of left ventricle at end diastole
LVIDs	- Internal dimension of left ventricle at end systole
LVM	- Left ventricle mass
LVPWd	- Left ventricle posterior wall thickness at end diastole
MBq	- Megabecquerel
mCi	- Millicurie
min	- Minutes
MOA	- Mono amine oxidase
ms	- Milliseconds
MUGA	- Multigated radionuclide ventriculography
MVO	- Mitral valve opening
NE	- Norepinephrine
NRG	- Neuregulin

NT-proBNP	- N-terminal pro brain natriuretic peptide
NYHA	- New York Heart Association
p.i.	- Post injection
PER	- Peak ejection rate
PFR	- Peak filling rate
p-gp	- P-glycoprotein
PS	- Phosphatylidserine
PV S/D ratio	- Pulmonary vein systolic to diastolic peak flow velocity ratio
RIC	- Radiation-induced cardiotoxicity
ROI	- Region of interest
ROS	- Reactive oxygen species
s	- Seconds
SD	- Systolic dysfunction; standard deviation
SDS	- Standard deviation score
sFlt-1	- Soluble Fms-like tyrosine kinase 1
Sm ROI	- Small left ventricle region of interest
SPECT	- Single photon emission computed tomography
TAC	- Taxanes, Adriamycin, Cyclophosphamide
TIC	- Trastuzumab-induced cardiotoxicity
TNF- α	- Tumor necrosis factor α
Top2	- Topoisomerase II
t-PER; TPER	- Time to peak ejection rate
t-PFR; TPFR	- Time to peak filling rate
TUNEL	- Deoxynucleotidyl transferase biotin-dUTP nick-end labeling
UR	- Upper border
VMA	- Vanillylmandelic acid
VOI	- Voxel of interest
WB	- Western Blot
WH ROI	- Whole heart region of interest
WO	- Washout

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Curriculum vitae in English and Dutch

Ben Bulten was born on May 14th 1985 in Toldijk, a little village in the eastern part of the Netherlands (the 'Backcorner'). After enjoying a wonderful youth and finishing the Gymnasium at the Baudartius College in Zutphen, he moved to Nijmegen to study Medicine. He obtained his medical degree in 2010, after he was lured into the department of Nuclear Medicine for a one-month internship. The sheer beauty of this discipline made him choose to become a Nuclear Medicine physician. As a resident he first worked at the department of Internal Medicine in the Slingeland Ziekenhuis Doetinchem, after which he continued his residency at the department of Nuclear Medicine of the Radboudumc. During this residency, mainly in his spare time, he took the opportunity to partly perform the research that resulted in this thesis. After finishing his residency in 2014, he started at the University of Twente to complete his PhD, combining it with clinical Nuclear Medicine practice at different institutions.

Currently, Ben is working at the Streekiekenhuis Koningin Beatrix in Winterswijk and the Slingeland Ziekenhuis (again). He is married to Stieny, for over four years now, and they have recently moved to Ben's elderly home, which is scheduled for a major refurbishment in the coming years.

Ben Bulten werd op 14 mei 1985 geboren in Toldijk, een klein dorpje in de Achterhoek. Na een heerlijk onbezorgde jeugd en het behalen van zijn Gymnasium diploma aan het Baudartius College te Zutphen, verhuisde hij naar Nijmegen voor de studie Geneeskunde. Hij haalde zijn bul in 2010, nadat hij de afdeling Nucleaire Geneeskunde van het Radboud was binnengelokt voor een keuze co-schap. De pracht van dit kleine discipline deed hem besluiten nucleair geneeskundige te worden. Als arts-assistent werkte hij eerst anderhalf jaar op de afdeling Interne Geneeskunde van het Slingeland Ziekenhuis Doetinchem, waarna hij zijn opleiding tot nucleair geneeskundige voortzette in het Radboudumc. Gedurende de opleiding, grotendeels in zijn vrije tijd, begon hij aan het onderzoek dat heeft geresulteerd in dit proefschrift. Na het afronden van zijn opleiding in 2014, zette hij zijn promotietraject voort aan de Universiteit van Twente en combineerde dit met werk als (waarnemend) nucleair geneeskundige in diverse instellingen.

Momenteel werkt Ben in het Streekiekenhuis Koningin Beatrix te Winterswijk en (wederom) het Slingeland Ziekenhuis. Hij is getrouwd met Stieny, nu meer dan vier jaar, en samen zijn zij recent verhuisd naar Bens ouderlijk huis, welke in de komende jaren grondig gaat worden verbouwd.

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