Cardiac Function Assessed by Magnetic Resonance Imaging and Circulating Biomarkers during Adjuvant Breast Cancer Therapy:

Effect of Concomitant Neurohormonal Blockade

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Selected Abbreviations

ACEIs	Angiotensin-converting enzyme inhibitors
ARB	Angiotensin receptor blockers
b-SSFP	Balanced steady-state free precession
BNP	B-type natriuretic peptide
cTn	Cardiac troponins
CMR	Cardiovascular magnetic resonance
ECV	Extracellular volume
EF	Ejection fraction
EOS	End-of-study
FEC	5-fluorouracil, epirubicin and cyclophosphamide
GLS	Global longitudinal strain
HER-2	Human epidermal growth factor receptor-2
hs	High sensitivity
LGE	Late gadolinium enhancement
ITT	Intention to treat
LV	Left ventricular
MOLLI	Modified Look-Locker inversion recovery
PRADA	Prevention of cardiac dysfunction during adjuvant breast cancer therapy
ROI	Region of interest

List of Publications

Paper I

Rationale and Design of the Prevention of Cardiac Dysfunction during Adjuvant Breast Cancer Therapy (PRADA) Trial.

Heck SL, Gulati G, Ree AH, Schulz-Menger J, Gravdehaug B, Røsjø H, Steine K, Bratland Å, Hoffmann P, Geisler J, Omland T.

Cardiology. 2012;123(4):240-7.

Paper II

Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 x 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol.

Gulati G, Heck SL*, Ree AH, Hoffmann P, Schulz-Menger J, Fagerland MW, Gravdehaug B, von Knobelsdorff-Brenkenhoff F, Bratland Å, Storås TH, Hagve TA, Røsjø H, Steine K, Geisler J, Omland T.

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Paper III

Effect of candesartan and metoprolol on myocardial tissue composition during anthracycline treatment: the PRADA-trial

Heck SL, Gulati G, Hoffmann P, von Knobelsdorff-Brenkenhoff F, Storås TH, Ree AH, Gravdehaug B, Røsjø H, Steine K, Geisler J, Schulz-Menger J, Omland T.

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Paper IV

Neurohormonal Blockade and Circulating Cardiovascular Biomarkers During Anthracycline Therapy in Breast Cancer Patients: Results from The PRADA Study

Gulati G, Heck SL, Røsjø H, Ree AH, Hoffmann P, Hagve TA, Norseth J, Gravdehaug B, Steine K, Geisler J, Omland T.

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1. Introduction

Breast cancer is the most common cancer among women worldwide, affecting an estimated 1.7 million new women each year. It is also the leading cause of cancer death in women, with an estimated mortality of 522 000 in 2012.[1] Improved detection and contemporary treatment with surgery and adjuvant therapy has significantly improved survival. In an aging population this leads to an increasing number of cancer survivors [2] who are at risk of suffering from long-term side effects of cancer treatment.[3,4]

1.1. Heart failure

Heart failure is a clinical syndrome characterized by symptoms such as ankle swelling, dyspnea and fatigue, and signs such as edema and pulmonary rales, caused by structural or functional cardiac abnormalities.[5] This definition depends on clinical symptoms, but patients may have asymptomatic cardiac dysfunction. These patients are at increased risk of developing heart failure, and may benefit from treatment.[6,7] Left ventricular ejection fraction (LVEF) is a measurement of systolic function, calculated as the percentage of blood expelled from the left ventricle on each contraction (i.e., stroke volume divided by end-diastolic volume).[8]

1.2. Cardiotoxicity

Several of the therapeutic agents that have increased life expectancy among cancer patients may harm the heart.[4] At the same time, improvements in cardiovascular prevention and care has led to decreased age-adjusted cardiovascular mortality,[9] and in an aging population there is an increase in patients with cancer and co-existing cardiovascular risk factors and disease. Cardiotoxicity may limit cancer treatment and reduce life quality, and this concern is the basis of the rapidly evolving field of cardio-oncology.[4,10]

There are several definitions of cardiotoxicity. The National Cancer Institute (USA) broadly defines it as "toxicity that affects the heart".[11] In clinical trials and daily practice, cardiotoxicity has usually been defined as a decline in LVEF to below a defined normal range, where threshold values differ between definitions. Some definitions also take the presence of clinical symptoms into account. An early definition of anthracycline cardiotoxicity was decline in LVEF > 10% to final LVEF < 50% as assessed by nuclear imaging.[12] According to the definition of the Cardiac Review and Evaluation Committee supervising trastuzumab

clinical trials, cardiac dysfunction is confirmed when one or more of the following criteria are fulfilled: 1) cardiomyopathy with decrease in LVEF that was either global or more severe in the septum; 2) symptoms of congestive heart failure (CHF); 3) associated signs of CHF, including but not limited to S3 gallop, tachycardia, or both; and 4) decline in LVEF of at least 5% to less than 55% with accompanying signs or symptoms of CHF, or a decline in LVEF of at least 10% to below 55% without accompanying signs or symptoms.[13] Recently, expert consensus from the American Society of Echocardiography and the European Association of Cardiovascular Imaging defined cancer therapy related cardiac dysfunction as decrease in the LVEF of >10 percentage points, to a value <53%, basing the threshold value on the normal reference value for 2D echocardiography.[8,14] Other subclinical signs of myocardial damage, such as release of circulating cardiac biomarkers [15] may precede a decrease in LVEF, and to date there is no universally accepted definition of cardiotoxicity.[10]

1.3. Cardiotoxicity in breast cancer treatment

The choice of treatment of breast cancer depends on a number of factors, mainly stage, grade and receptor status. There are significant differences in treatment between patients with early breast cancer, where the treatment goal is curative, and metastatic breast cancer, where the goal is to reduce symptoms and prolong life. The focus in this thesis is on patients with early breast cancer, and treatment of metastatic cancer will not be discussed further.

Patients with early breast cancer are offered surgery with either breast-conservation or mastectomy. Further treatment after surgery depends on tumor characteristics and surgery type and may involve chemotherapy, radiotherapy, endocrine therapy, and treatment with the monoclonal antibody trastuzumab.

1.3.1. Anthracyclines

Anthracycline-containing chemotherapy significantly reduces breast cancer recurrence as well as breast cancer specific and overall mortality, and remains, despite known cardiotoxicity, a cornerstone in adjuvant treatment for early breast cancer.[16,17] Anthracyclines have since its discovery in the 1960s been among the most utilized antineoplastic drugs. Originally derived from the bacterium Streptomyces, they are highly effective, and have contributed significantly to increased survival in patients with various cancer types.[18,19] However, it soon became apparent that anthracyclines could cause serious heart damage. The cardiotoxicity was found to be related to cumulative dose. Doxorubicin, an

anthracycline introduced in the 1970s, is used in treatment of various hematological and solid cancers. In 1979, von Hoff reported that at life-time doses of 400, 550 and 700 mg/m² of doxorubicin, the prevalence of congestive heart failure was 3, 7 and 18 %, respectively.[20] In an analysis from 2003, Swain et al estimated the risk to be somewhat higher, and that 5, 26 and 48% of patients experienced cardiac events at 400, 550 and 700 mg/m² cumulative doses of doxorubicin.[21] The cumulative dose versus heart failure curve is not linear, and at doses less than 300 mg/m², few patients will develop cardiac problems, and the slope of the curve steepens at doses exceeding 4-500 mg/m² (Figure 1). [19,21] There are, however, other factors that modify the risk of cardiotoxicity. Administration schedule matters, and lowering the peak doses through more frequent administrations or continuous infusions reduces the risk of cardiotoxicity without diminishing the antineoplastic effect.[22,23] Over the years, advances have been made to reduce cardiotoxicity. Liposome-encapsulated compounds [24] as well as the semisynthetic anthracycline epirubicin [25] have been shown to be less cardiotoxic than their parent compound, and early studies showed that the median dose to develop signs of cardiotoxicity was 468 mg/m² of doxorubicin, compared to 935 mg/m² of epirubicin.[26] Yet, not all patients who receive cumulative doses in the high range develop heart failure, and some patients experience cardiotoxicity at low cumulative doses. Identified predisposing factors include extremes of age, previous chest irradiation, severe co-morbidities or underweight as well as coexisting cardiovascular risk factors such as diabetes, hypertension or obesity.[20,27-29]

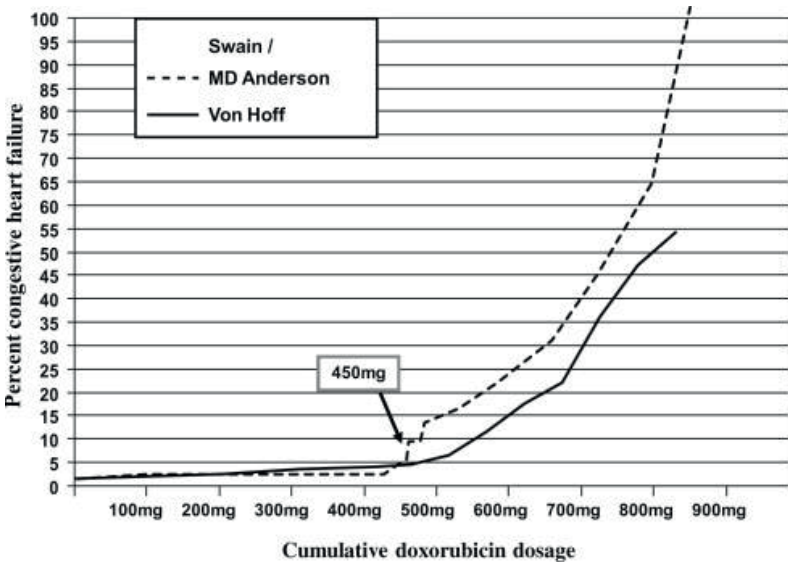

Figure 1
The relationship between cumulative dose and risk of heart failure.
Reprinted from Ewer MS, Von Hoff DD, Benjamin RS. A historical perspective of anthracycline cardiotoxicity. Heart Fail Clin.2011; 7(3):363-72., with permission. Copyright© 2011, Elsevier[19]

The cardiotoxic effect of anthracyclines, classified as type I, is considered irreversible.[20,30-32] Recent findings implicate inhibition of the enzyme topoisomerase 2β as an important mediator of anthracycline cardiotoxicity.[33,34] Topoisomerases are nuclear enzymes essential to DNA replication, transcription and recombination and exists in two isoforms. Topoisomerase 2α is abundant in rapidly proliferating cells such as tumor cells, whereas topoisomerase 2β is expressed in quiescent cells like cardiomyocytes. Anthracyclines target topoisomerase 2, and exert their anti-neoplastic effect by inhibiting topoisomerase 2α in tumor cells. In the myocardium, by inhibiting topoisomerase 2β, anthracyclines cause cell death by inducing DNA double-stranded breaks and activation of the apoptotic pathway. Also, anthracyclines cause topoisomerase 2β dependent mitochondrial dysfunction and energy depletion as well as generation of reactive oxygen species that further damage cardiomyocytes (Figure 2).[33-35] Typical histologic changes are myofibrillar disarray and loss, cytoplasmic vacuolization, myocyte loss due to both apoptosis and necrosis, and interstitial edema and fibrosis.[36-39]

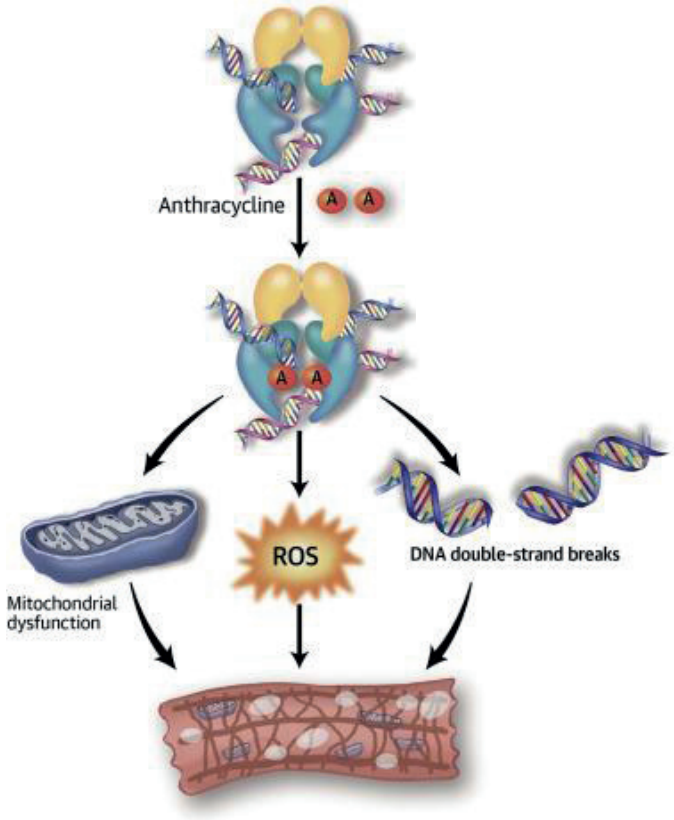

Figure 2
Doxorubicin inhibits topoisomerase 2β, causing double-stranded DNA breaks, mitochondrial dysfunction and increase in reactive oxygen species (ROS), resulting in myofibrillar disarray and vacuolization and cardiomyocyte loss.

Adapted from Vejpongsa P, Yeh ET. Prevention of anthracycline-induced cardiotoxicity: challenges and opportunities. J Am Coll Cardiol. 2014;64(9):938-45 Copyright© 2014 American College of Cardiology Foundation with permission from Elsevier.[35]

1.3.2. 5-Fluorouracil

5-Fluorouracil is an antimetabolite used with epirubicin in the 5-fluorouracil, epirubicin and cyclophosphamide (FEC) regimen, and patients receive 600 mg/m² per FEC cycle. It has been associated with vasospasms and angina-like chest pain, and rarely, arrhythmias, myocardial infarction, cardiomyopathy and sudden cardiac death. Cardiotoxicity usually occurs early in the treatment and is generally reversible. Pre-existing coronary heart disease, continuous infusion and doses exceeding 800 mg/m² are associated with higher risk of cardiotoxicity.[40,41]

1.3.3. Cyclophosphamide

Cyclophosphamide, the "c" in the FEC regimen, is an alkylating agent that inhibit DNA replication and thereby protein synthesis.[28] Like 5-Fluorouracil, symptoms of cardiotoxicity usually develops within the first weeks administration. Cyclophosphamide metabolites are believed to damage endothelial cells and cause direct myocardial damage, and may lead to acute cardiomyopathy, hemorrhagic myocarditis and heart failure.[4] Doses exceeding 1.55 g/m² per day may cause significant cardiotoxicity. The cyclophosphamide dose per FEC cycle in adjuvant treatment for early breast cancer is 600 mg/m², and cyclophosphamide cardiotoxicity at these doses is rarely a problem.[42,43]

1.3.4. Taxanes

Taxanes are antineoplastic drugs widely used in breast cancer therapy. They inhibit cell division by binding to the microtubules, which are essential in mitosis, thereby inhibiting cell division. Taxanes may cause bradycardia, conduction anomalies and heart block, and may potentiate the cardiotoxic effect of anthracyclines, especially with high anthracycline doses.[28,44,45] However, in modern adjuvant breast cancer regimens, taxanes do not increase anthracycline cardiotoxicity.[46,47]

1.3.5. Trastuzumab

Trastuzumab is a monoclonal antibody against the transmembrane receptor tyrosine kinase HER2. Overexpression of this protein, which occurs in about 25% of breast cancer patients, is associated with more aggressive cancer and poorer prognosis, and treatment with trastuzumab significantly reduces breast cancer recurrence and improves survival in HER2 positive breast cancer patients.[48-50] Trastuzumab cardiotoxicity is not dose-dependent, is in many cases reversible on cessation and is responsive to heart failure therapy.[30,51,52] Also, trastuzumab may often be reintroduced after a pause without renewed cardiac problems.[52] As opposed to anthracyclines, trastuzumab cause little or no histopathological changes in the myocardium.[30,52,53] The pathophysiological mechanisms are not fully elucidated, but it may be that trastuzumab on binding to HER2 inhibits intracellular pathways, ultimately causing mitochondrial membrane depolarization, ATP depletion and contractile dysfunction. This may explain the observed contractile dysfunction without significant cardiomyocyte ultrastructural changes.[54] In the heart, HER2 is involved in signaling that promotes cardiomyocyte growth, repair and survival.[30] Trastuzumab binds to the extracellular domain of HER2, thereby blocking signaling pathways essential for myocardial protection and function.[4,30] Patients subjected to either concurrent or previous anthracycline treatment are especially vulnerable to trastuzumab induced cardiac dysfunction, likely because trastuzumab inhibits repair processes of cells injured by anthracyclines. In a pivotal study from 2001, 27% of patients who had received trastuzumab concurrently with anthracyclines developed symptomatic or asymptomatic cardiac dysfunction, and 16% developed symptomatic heart failure.[55] More recent studies show that risk of cardiotoxicity is less when trastuzumab is given some time after anthracycline exposure. In these studies, about 7-19% of the patients developed asymptomatic cardiac dysfunction, whereas 2-3% developed symptomatic heart failure.[4,48,56] Other identified risk factors are advanced age, overweight, a history of heart disease and low pre-treatment LVEF.[29,54,56-58]

1.3.6. Radiotherapy

Radiotherapy may affect the heart in several ways. Ionizing radiation cause micro- and macrovascular damage and affect both the pericardium and heart valves. The clinical cardiotoxic effects of radiotherapy usually manifest years after exposure and include ischemic heart disease due to accelerated coronary artery disease, diffuse fibrosis and restrictive cardiomyopathy, valve regurgitation

or stenosis as well as pericarditis and pericardial constriction.[59,60] The risk of radiation-induced heart disease increases with left sided irradiation and with a high cumulative irradiation dose. A large Scandinavian population based study from 2013 showed that radiotherapy linearly increased the rate of major coronary events with 7% per Grey mean dose to the heart.[61] Other known risk factors are young age, concomitant anthracycline treatment, cardiovascular risk factors and pre-existing cardiovascular disease.[61] Advances in radiotherapy for early breast cancer such as improved treatment planning and deep inspiration breath hold technique all reduce the radiation dose to the heart and will likely contribute to lessen the risk of radiotherapy-induced heart disease.[61-63]

1.3.7. Hormonal therapy

For patients with estrogen receptor positive disease, treatment with estrogen receptor blockers or estrogen-lowering aromatase-inhibitors reduce breast cancer recurrence and improve survival.[64] Estrogen exerts beneficial effects on the female cardiovascular system, including slowed development of atherosclerosis and cardiac hypertrophy as well as favorable effects on the lipid profile.[65,66] Aromatase inhibitors have been shown to increase serum cholesterol and is associated with a small increase in cardiovascular risk, whereas this seems not to be the case with estrogen receptor blockers.[46]

1.4. Detection of cardiotoxicity

Cardiotoxicity range from subclinical, histological changes to symptomatic heart failure, and the available methods of detection have different strengths and limitations. Selection of the most appropriate method depends on a number of factors, including what stage of cardiotoxicity you want to detect, the level of precision desirable, and resources available. The myocardium has contractile reserves, and a decline in function, either due to irreversible cell damage or reversible myofibrillar dysfunction, will only be detectable after these resources have been exhausted. Simply measuring LVEF may therefore underestimate the myocardial damage. Earlier signs of myocardial damage may be detectable as change in myocyte ultrastructure by endomyocardial biopsy or by measuring circulating biomarkers (Figure 3).[4,67]

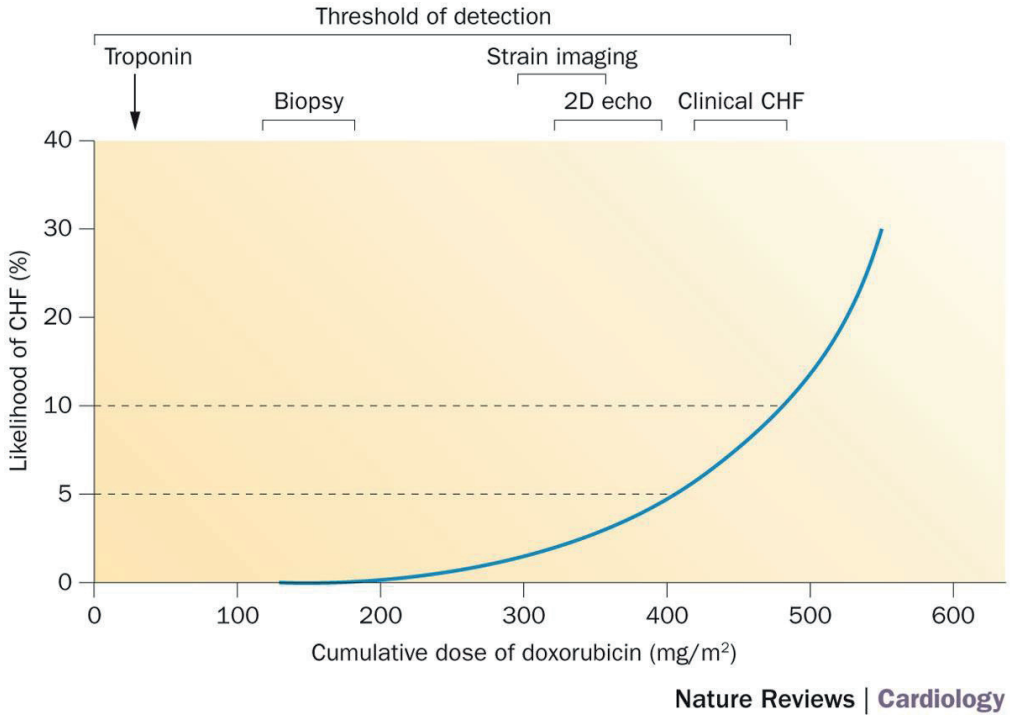

Figure 3

Different methods have different thresholds of detection of cardiotoxicity. CHF denotes congestive heart failure.

Reprinted from Ewer MS, Ewer SM. Cardiotoxicity of anticancer treatments. Nat Rev Cardiol. 2015;12(9):547-58 with permission. Copyright© 2015 Nature Publishing Group [4]

1.4.1. Left ventricular function

1.4.1.1. Cardiovascular MRI

Cardiovascular MRI (CMR) is the reference standard method to quantify ventricular dimensions and systolic function.[68] It is non-invasive, and without ionizing radiation.[69-71] Typically, balanced steady-state free precession (b-SSFP) images are used for these quantifications. These sequences have short acquisition times, and provide excellent contrast between blood in the ventricular cavities and the myocardium, as well as good contrast between the myocardium and pericardial fat, and facilitate reliable delineation of contours and precise measurements of ventricular volumes and mass.[72,73] Although improvements to automate detection have been made, manual delineation or rigorous control and correction of automated contours are still necessary to assure accuracy.[74,75] Image planes may be planned in any direction, and contiguous stacks of cine loops provide whole heart coverage, and volumes and mass may be calculated without geometric assumptions.[74,76] Image analysis is usually performed on commercially available software by drawing left ventricular endo-and epicardial contours and right ventricular endocardial contours at end-diastole and end-systole, typically in contiguous short axis stacks (Figure 4).[75] Numerous studies have shown that the accuracy of CMR for ventricular volumes and mass is superior to other imaging modalities such as two dimensional (2D) echocardiography and nuclear imaging.[68] Moreover, CMR has a low intra-observer, inter-observer and inter-study variability. By using CMR in clinical trials, sample size may be reduced significantly, which makes CMR an attractive tool.[69,77-79] However, there are also disadvantages to CMR. CMR scanners are expensive and not abundantly available. CMR imaging is a complex procedure, and requires extensive training of dedicated radiographers as well as physicians. Duration of the examination may be an issue both in managing limited timeslots on the MRI scanner, as well as for patients prone to claustrophobia. Many cardiac devices are incompatible with MRI scanning. Also, patient-related factors such as arrhythmias, dyspnea or obesity may reduce imaging quality.[77]

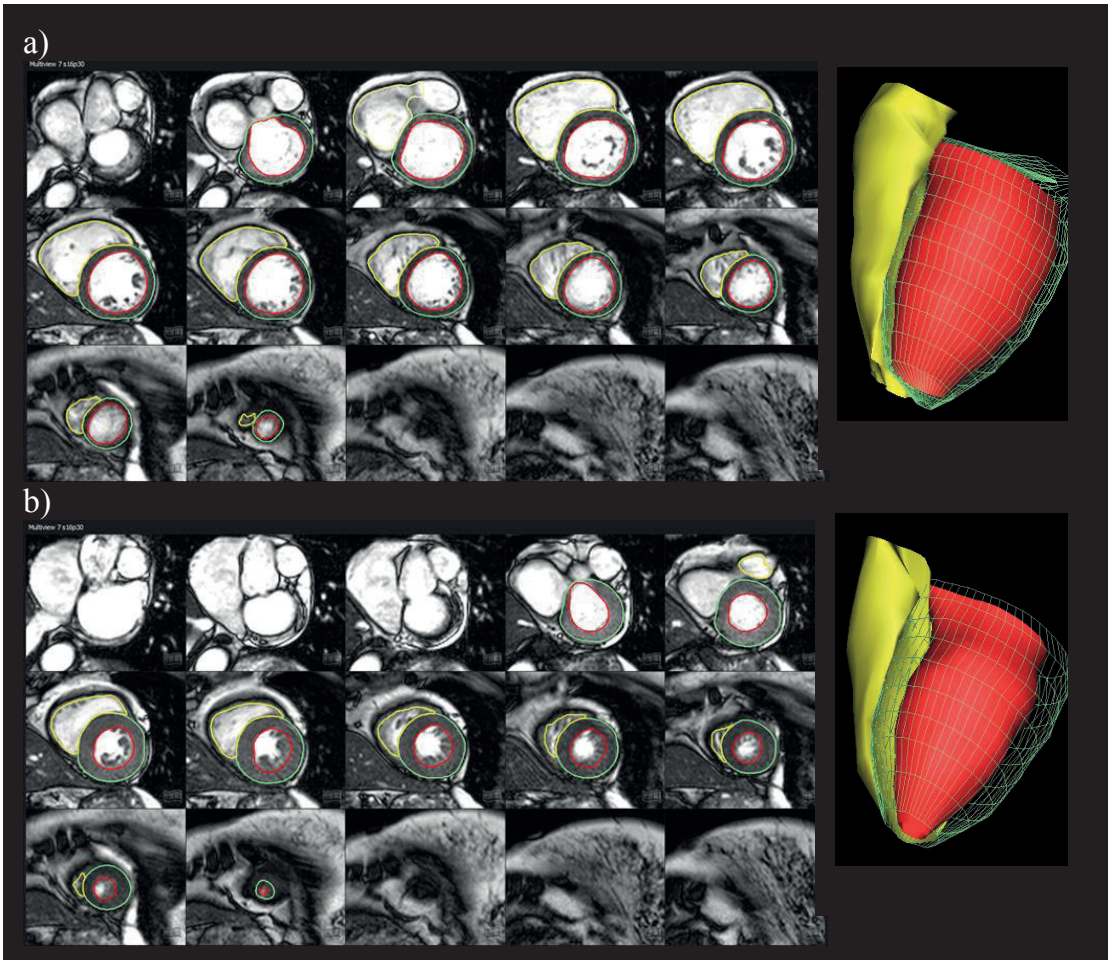

Figure 4
Summation of discs method: In contiguous short axis slices covering the entire ventricle, left (red) and right (yellow) endocardial and left epicardial (green) contours are delineated in diastole (a) and systole (b).

Ventricular volumes are calculated as the sum of the cross-sectional areas of each slice multiplied by the slice thickness.

1.4.1.2. Echocardiography

Echocardiography is a widely available, non-invasive method that poses no risk to the patient, and the most common way of measuring systolic function in daily clinical routine. 2D echocardiography provides tomographic images in standardized orientations. LVEF measurements are based on geometrical assumptions, and are significantly less accurate and reproducible than measures by CMR.[79] Newer echocardiographic techniques are more sensitive to changes in systolic function. Technological advances have made real-time three dimensional (3D) echocardiography imaging feasible, which allow for full volume acquisitions and measurements of ventricular volumes and ejection fraction independent of geometrical assumptions. The accuracy of these measures is higher than with 2D echo, and comparable to CMR, but varying imaging quality and echocardiographer experience lead to higher variability.[80,81]

Echocardiographic strain measures tissue deformation, and strain rate speed of tissue deformation. These methods provide information about active myocardial movement in radial, circumferential or longitudinal directions, and are used as sensitive measures of systolic function and regional myocardial dysfunction, and studies have indicated that strain is a sensitive marker of early cardiotoxicity during anthracycline treatment.[82,83] Strain is unit-less, and is usually expressed as percent change in the length of a myocardial segment, and shortening is expressed as negative strain. 2D speckle-tracking echocardiography is a technique where small acoustic markers in the myocardium called speckles are tracked. Shift in these speckles represent movement, and strain and strain rate may be calculated. Speckle-tracking echocardiography is largely angle independent, but requires high quality images with both high temporal and spatial resolution.[84,85]

Chemotherapy and radiotherapy may also cause impaired ventricular relaxation and diastolic dysfunction.[86,87] Although there are methods to evaluate diastolic function by CMR, these are not widely used, and echocardiography is the preferred means of quantification.[88,89] The relationship of early transmitral velocities (E) to late transmitral velocities (A) and peak mitral annular velocity during early filling (e'), expressed as the ratios E/A and E/e', are measures used to examine diastolic function.[89]

1.4.2. Tissue composition

As cardiac dysfunction due to cardiotoxicity becomes apparent only after cardiac reserves have been exhausted, means of early detection are needed. There is significant inter-individual variability in tolerance to adjuvant therapy. Myocardial damage may occur as early as after first exposure, and recognition of asymptomatic changes in tissue composition might help identify patients at risk.[4,19]

1.4.2.1. Histopathology

Endomyocardial biopsy has been an important tool in diagnosing cardiotoxicity, and was used in the 1980s to establish whether patients would tolerate additional anthracycline treatment. Also, it has provided valuable information on the cardiotoxic effect of different chemotherapeutic regimens.[19] However, it is an invasive and costly procedure, not without risk to the patient and not available everywhere. With the evolution of less cardiotoxic regimens and better non-invasive imaging techniques, endomyocardial sampling is rarely used.[8,90]

1.4.2.2. CMR

In addition to accurate measures of cardiac systolic function, CMR offers a range of sequences that visualize myocardial morphological changes. Myocardial edema may be identified on T2 STIR images, either as focal hyperintensities, typical in acute myocardial infarction, or as globally increased T2 ratio of the myocardium to skeletal muscle, as in acute myocarditis.[91] Late gadolinium enhancement (LGE) imaging is an excellent technique for detection of focal fibrosis and is widely used to assess different ischemic and non-ischemic cardiomyopathies. After injection, extracellular gadolinium contrast will in healthy myocardium distribute evenly in the extracellular matrix surrounding densely packed cardiomyocytes. In LGE images, typically acquired 10-20 minutes after contrast injection, the normal myocardium is nulled by inversion recovery pulses, and appears black. In conditions with expanded extracellular space and altered wash-in and wash out kinetics such as in post-infarction collagenous scars or in necrotic tissue with myocyte membrane rupture, gadolinium contrast will accumulate. On LGE images these areas will appear bright.[92,93] However, LGE imaging depends on the difference between healthy and fibrotic myocardium and fails in depicting diffuse myocardial changes.[94] In diffuse myocardial fibrosis, the extracellular space is expanded by collagen accumulation, and this is a common denominator in many cardiomyopathies,

including anthracycline and radiotherapy induced cardiomyopathies. These diffuse changes may be invisible in conventional LGE images, and although there have been some reports of LGE in chemotherapy-induced cardiac injury,[95-97] more recent findings indicate that LGE is not a common finding.[98-101]

Newer mapping techniques are emerging as useful tools in quantifying diffuse myocardial changes that were previously inaccessible to non-invasive imaging. Key methods are T1 mapping and the derived extracellular volume (ECV) fraction.

T1 mapping

Signal intensity in conventional CMR images are set on an arbitrary scale that vary from one examination to another, and signal measurement cannot be used to quantify pathology.[102] T1 mapping techniques circumvent this problem. When placed in an MRI scanner, tissue protons become magnetized in the longitudinal direction. By applying a radiofrequency pulse, the magnetization of the protons can be tilted, and as the pulse ceases, the protons will realign with the longitudinal magnetic field, at an exponential rate. T1 is defined as the time, measured in milliseconds, required for 63% of the longitudinal magnetization to recover. T1 depends on the environment of the proton, and varies from tissue to tissue. Thus, T1 mapping may be used to quantify tissue properties.[103,104] In T1 mapping, multiple images with different inversion times are acquired, and the images are combined to calculate the T1 of each voxel. To combine these images, the heart must be in the same position in each image, and T1 mapping is sensitive to motion.[94] The most validated myocardial T1 mapping technique is the ECG triggered Modified Look-Locker Inversion-recovery (MOLLI) sequence, described by Messroghli and colleagues,[105-107] but there are numerous different mapping schemes with different strengths and limitations.[104,108] The original MOLLI sequence consists of single breath-hold series with 3 inversions, the first two followed by 3 images and the last by 5, with one diastolic image per heartbeat, and 3 dummy heartbeats for magnetization recovery between inversions. This scheme may be referred to as 3(3)3(3)5 (Figure 5). T1 may be depicted on parametric maps, where the signal intensity of each voxel represents the T1 value of the corresponding myocardium.

Native T1 has been shown to be elevated in a number of conditions, including amyloidosis, hypertrophic and dilated cardiomyopathy and myocarditis, and decreased in Fabry disease as well as in iron overload.[109-113] However, the

measured T1 signal in each voxel comes from a mixture of cardiomyocytes and extracellular matrix. There is considerable overlap of values in health and disease, as well as vendor and magnetic field specific differences, and these factors may limit the clinical use of native T1 measurements.[108,114,115]

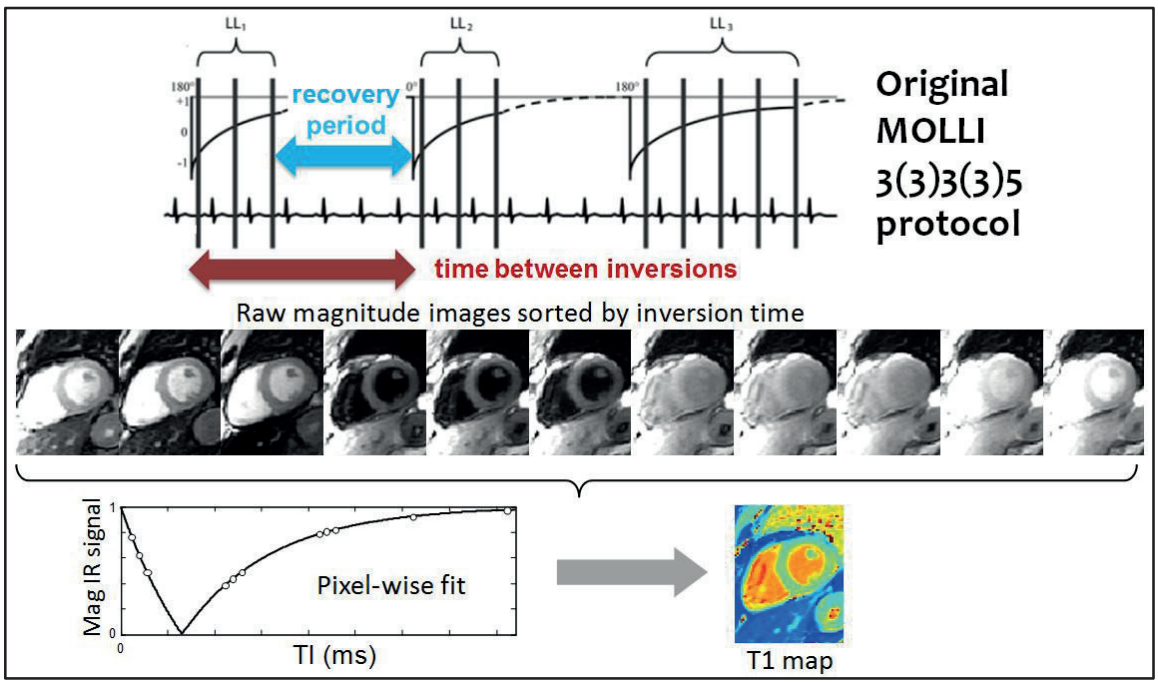

Figure 5
The MOdified Look-Locker Inversion Recovery (MOLLI) scheme for T1-mapping in the heart.
Reprint permitted from Kellman P, Hansen M. T1-mapping in the heart: accuracy and precision. J Cardiovasc Magn Reson. 2014;16(1):2 © Kellman and Hansen; licensee BioMed Central Ltd. 2014 [116]

ECV fraction

Many cardiac diseases lead to expansion of the extracellular space, through edema or fibrosis. Gadolinium contrast is an extracellular contrast medium that shortens T1 relaxation times, and the relationship between gadolinium concentration and $\frac{1}{T1}$ is assumed to be linear in conventional analysis. After injection, gadolinium will distribute evenly in the extracellular space, and voxels with expanded extracellular space will have more gadolinium molecules and therefore shorter T1 relaxation times (i.e. lower T1 values) than voxels containing densely packed cardiomyocytes. Post-contrast T1 may thus say something about the extent of extracellular space. However, post-contrast T1 measurements are very sensitive to measurement time-point as well as individual variations in gadolinium kinetics. After injection, gadolinium penetrates the extracellular space quickly, and clears slowly from both tissue and blood, and there will be an approximate equilibrium between plasma and the myocardial extracellular space 12-50 minutes after a bolus injection.[117] Blood cell and plasma volume fractions may be obtained by measuring hematocrit. By measuring native and post-contrast T1 in blood and in the myocardium as well as hematocrit, an estimate of the myocardial ECV fraction may be calculated (Figure 6). Fifteen minutes is a recommended and validated delay.[108,114,117] ECV fraction has been shown to correlate strongly with histologically determined interstitial fibrosis.[118,119] There is a wide range of normal values and overlap between health and disease, and even though group differences between patients and controls have been documented in numerous publications, setting a cut-off value is challenging. Still, the reproducibility of ECV fraction measurements makes it an interesting biomarker in longitudinal studies.[120-123] ECV fraction provides information about the relative distribution of extracellular matrix and cellular volume. However, it is a relative measure, and if ECV fraction increases over time, this may be due to either extracellular space expansion by edema or fibrosis, or it may be caused by reduced cellular volume due to cell shrinkage or cell loss. Recently, the derived parameters total cellular volume and total ECV have been introduced in an effort to overcome this limitation. Total myocardial volume may be calculated by dividing mass by the myocardial specific density 1.05 g/ml. The total myocardial ECV and the total cellular volume are then simply ECV fraction and (100% - ECV fraction %) multiplied by the total myocardial volume, respectively. This may prove especially valuable in longitudinal studies.[123-125]

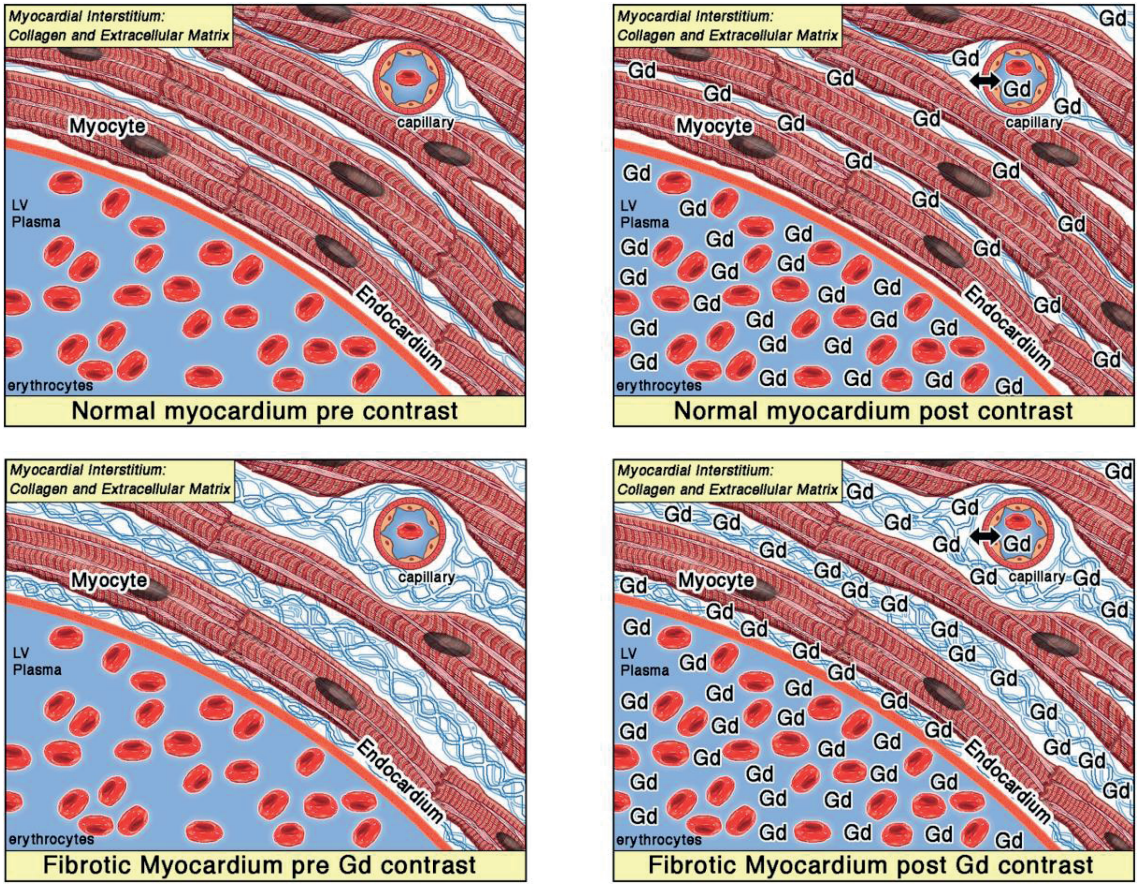

Figure 6
Myocardial ECV fraction may be estimated by measuring native and post contrast T1 in the myocardium and blood, as well as hematocrit**.**

$$\text{ECV fraction \%} = (1 - \text{hct}) * \frac{\frac{1}{T1myo_{postcontrast}} - \frac{1}{T1myo_{native}}}{\frac{1}{T1\text{blood}_{postcontrast}} - \frac{1}{T1\ blood_{native}}}$$

Adapted from Schelbert EB, Fonarow GC, Bonow RO, Butler J, Gheorghiade M. Therapeutic Targets in Heart Failure. J Am Coll Cardiol. 2014;63(21):2188-98 © 2014, American College of Cardiology Foundation, with permission from Elsevier [126]

1.4.3. Circulating cardiac biomarkers

Multiple studies explore the relationship between cancer therapies and circulating cardiac biomarkers. These studies have various designs and encompass different cancer entities and treatment regimens, and patients with different risk profiles. Also, timing of blood sampling, choice of biomarkers, as well as biomarker assay sensitivity contribute to inconsistent results.[127,128]

1.4.3.1. Troponins

Cardiac troponins (cTn) I and T are components of the cardiomyocyte myofibrillar contractile apparatus, and are released on myocardial cell death. Circulating cTns are sensitive markers of myocardial injury, and are used for the diagnosis of myocardial infarction and risk stratification in acute coronary syndrome. Recently, highly sensitive assays (hs) that detect very low circulating cTn levels have been introduced. Detectable cTns are also associated with adverse outcome in a number of conditions such as stable coronary disease, heart failure as well as structural heart disease and risk of all-cause mortality in the general population.[129-131] Moreover, increased cTns have been associated with reduction in LVEF as well as risk of future cardiac events in patients treated with high dose chemotherapy.[15,132] There have also been studies showing increased cTns preceding fall in ejection fraction during trastuzumab therapy, although this seems generally to be related to previous exposure to anthracyclines.[133,134] Increased cTns pre trastuzumab has been identified as a risk factor for trastuzumab cardiotoxicity, consistent with the theory that trastuzumab inhibits repair of damaged and vulnerable cells.[127,133-136] The predictive ability of hs cTns during treatment with low-dose anthracyclines and contemporary radiotherapy is less well elucidated.[127,128]

1.4.3.2. Natriuretic peptides

Natriuretic peptides are important biomarkers for the diagnosis and prognosis of heart failure. Stretching of the ventricles by pressure or volume overload induce synthesis of pre-proBNP, which is subsequently cleaved to proBNP, and further to the biologically active BNP and the inactive amino-terminal fragment NT-proBNP.[137] High-dose anthracyclines and radiotherapy have been associated with increased BNP and NT-proBNP,[127,138,139] whereas the effect of trastuzumab on natriuretic peptides is less clear.[127,134,140,141]

1.4.3.3. C-reactive protein

CRP is an acute phase protein produced by hepatocytes after inflammatory stimulus, and is principally regulated by interleukine-6. It is elevated by systemic inflammation and tissue injury. Inflammation is a risk factor for cardiovascular disease, and increased levels of hs-CRP are associated with risk of cardiovascular disease and adverse cardiovascular events.[142-144] hs-CRP has been shown to increase during anthracycline therapy,[134,141] and one study found that hs-CRP may predict subsequent decline in LVEF during trastuzumab therapy,[140] while other studies did not find this association.[141]

1.4.3.4. Galectin-3

Galectin-3 is a biomarker that seems to be a mediator in profibrotic pathways, and has been related to mortality in heart failure and in the general population.[145,146] Expressed in activated macrophages, it binds to fibroblasts and the extracellular matrix, and promotes cardiac fibroblast proliferation, collagen depositions and ventricular dysfunction. It has been proposed as a potential biomarker in the evaluation of cardiotoxicity, however sparse data is available, and no significant association between cancer therapy and galectin-3 has been established.[147]

1.5. Preventive measures

1.5.1. Adjusting adjuvant treatment

Over time, modifications of cancer therapy regimens have been made that reduce the risk of cardiac injury. The harmful effects of anthracyclines may be mitigated by reduced cumulative and peak doses, by less toxic analogs or by lipid encapsulation. The risk of combining anthracyclines with trastuzumab is reduced when given sequentially instead of concomitantly.[4] Contemporary radiotherapy techniques reduce delivered radiation to the heart.[62,63] Still, the problem of cardiotoxicity remains, and with the increasing number of cancer survivors in an aging population there is a need for additional preventive strategies.[4,148]

1.5.2. Exercise

There is some preclinical evidence from animal models that aerobic exercise might attenuate anthracycline as well as trastuzumab cardiotoxicity.[148,149] This is an appealing strategy because it has been shown to be well tolerated and to improve other cancer therapy related problems such as fatigue and quality of life. Structured exercise reduces underlying cardiovascular risk factors, and may suppress oxidative stress and systemic inflammation. However, evidence from clinical trials data is sparse.[150,151]

1.5.3. Cardioprotective medication

1.5.3.1. Dexrazoxane

Dexrazoxane has been shown effective in reducing anthracycline cardiotoxicity. Originally thought to exert its protective effect through scavenging of free radicals, recent evidence indicates topoisomerase II β inhibition.[4,152] Although the evidence is not strong, concern that dexrazoxane might attenuate the oncologic efficacy of anthracyclines has limited its use in adult populations.[4,153,154]

1.5.3.2. Statins

As reactive oxygen species is considered part of the anthracycline cardiotoxicity mechanisms, the attenuating effect of statins on vascular inflammation and oxidative stress has promoted the idea that statins may mitigate the harmful effects on the heart. Some preliminary studies support this notion, and several larger, placebo-controlled trials are underway to assess this.[155-160]

1.5.3.3. Angiotensin converting enzyme inhibitors/ angiotensin receptor blockers

The renin-angiotensin-aldosterone system (RAAS) is a complex neuroendocrine system, and a detailed description of its regulation is beyond the scope of this thesis. Triggered by decreased arterial blood pressure, decreased sodium chloride load in the renal distal tubules and sympathetic activation, RAAS activation and subsequent increased angiotensin II levels have pleotropic effects. These include vasoconstriction, sodium and water retention, myocyte hypertrophy as well as stimulation of myocardial fibrosis. RAAS activation plays a central role in the development of heart failure and adverse cardiac remodeling.[161-164] Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) reduce mortality and morbidity in heart failure, and have been shown to prevent progression in patients with symptomatic or asymptomatic left ventricular dysfunction.[7,165-168] Animal models indicate that the RAAS plays an important role in anthracycline cardiotoxicity, and that interruption of this pathway may attenuate myocardial damage.[169-172] Results from an open-label, controlled trial of 40 non-Hodgkin lymphoma patients [173], and a single-blinded, placebo controlled trial of 49 patients with various cancer entities [174,175] indicate that preventive angiotensin receptor blockade might limit anthracycline-induced cardiac dysfunction. In an open-labeled, randomized study of 114 patients with troponin increase after high-dose chemotherapy, early treatment with ACEIs prevented decline in LVEF and cardiac events.[176] However, these studies included patients with various cancer entities, risk factors and treatment regimens, and data from larger, randomized, placebo-controlled double-blind trials in homogenous patient populations is lacking. Although treatment with ACEIs or ARBs is recommended in patients with trastuzumab or radiotherapy induced cardiac dysfunction, little is known about the effect of preventive administration.[59,177]

1.5.3.4. Beta-blockers

Heart failure and decreased organ perfusion leads to adrenergic activation that in the short term leads to increased heart rate and contractility, vascular resistance and improved cardiac output. However, persistent adrenergic activation leads to increased metabolic demands on the failing heart, and contribute to further decline in cardiac function. Beta-blockers have for the past two decades been central in treatment of heart failure, and have been proven to improve myocardial function and significantly reduce both morbidity and mortality.[178,179] Results from animal models indicate that both selective [180] and non-selective [181] beta-blockade might attenuate anthracycline cardiotoxicity. Data from small randomized trials of beta–blockade during anthracycline therapy,[182] and combined ACE inhibition and beta-blockade during treatment of hematological malignancies [183] show beneficial effects on left ventricular systolic function. However, these trials encompassed patients with different cancer types and treatment regimens, and one trial was open labelled [183], the other single blinded.[182] Similar to ARBs and ACEI, data from larger, randomized, placebo-controlled double-blind trials in homogenous patient populations is needed.

2. Rationale and General Aims of the Thesis

Based on this background, the PRevention of cArdiac Dysfunction during Adjuvant breast cancer therapy (PRADA) trial was designed. Akershus University Hospital is primary hospital for about 10 % of Norway's population, and has large oncology and cardiology departments. Rebuilt in 2008, and equipped with modern imaging technology, the hospital seemed well suited for a trial on the prevention of cardiotoxicity during adjuvant breast cancer treatment.

The hypothesis of the study was that concomitant therapy with the angiotensin-receptor-blocker, candesartan, or the beta-blocker, metoprolol, would alleviate the left ventricular dysfunction and/or myocardial injury associated with adjuvant, anthracycline-containing regimens with or without trastuzumab and radiotherapy.

The general aims of this thesis are twofold:

- To assess the value of CMR indices and circulating biomarkers as indicators of cardiotoxicity
- To assess whether candesartan and/or metoprolol prevent myocardial injury and remodeling during adjuvant treatment for early breast cancer

3. Materials and Methods

3.1. Study design and participants

The PRADA trial was a 2 x 2 factorial, randomized, placebo-controlled, double-blind trial. Women aged 18-70 years, who after surgery for early breast cancer were scheduled to initiate anthracycline-containing adjuvant therapy, were eligible. Main exclusion criteria were prior anthracycline treatment or chest irradiation, serious concomitant illness, cardiac dysfunction, prior cardiovascular disease, renal failure, hypotension, hypertension, indication or contraindications for the study drugs and inability to undergo CMR. Between September 2011 and September 2014, 130 patients were included at Akershus University Hospital. Two patients were excluded because they did not receive adjuvant treatment as planned, one patient was discovered to have had a subclinical myocardial infarction around the time of randomization, and one patient had previously been treated with chest irradiation. That left 126 patients in the cohort. All patients received FEC. Thirty-five patients received FEC only, 63 patients had additional radiotherapy, 9 patients received additional trastuzumab only, and 19 patients received both radiotherapy and trastuzumab after completion of FEC.

3.2. Randomization and intervention

An independent statistician from Oslo Centre for Biostatistics and Epidemiology at Oslo University Hospital was responsible for randomization. A block randomization procedure was used, stratified for trastuzumab therapy. Since the cardiotoxic effects of taxanes, endocrine therapy and contemporary radiotherapy were expected to be relatively minor compared to the effects of anthracyclines and trastuzumab, no stratification according to these therapies was performed. When participants had signed the informed consent form, they were randomized to one of four treatment arms: Candesartan-metoprolol, candesartan-placebo, placebo-metoprolol and placebo-placebo. Placebos were identical in appearance to active tablets, and both study participants and personnel were blinded for treatment allocation. Patients started medication after baseline evaluation, and before commencement of adjuvant therapy. Starting dose was 8 mg and 25 mg daily for candesartan and metoprolol respectively. If well tolerated, the dose was increased stepwise to 32 and 100 mg. To assess compliance, remaining tablets were counted on every other visit during FEC treatment and every third visit during trastuzumab treatment and at the end of radiotherapy. In addition, all participants recorded tablet intake in diary.

3.3. Study visits

Study visits were scheduled before commencement of FEC (baseline examination), within two weeks of the first FEC cycle (visit 2), of completion of FEC (visit 3) and, for those concerned, of completion of radiotherapy and / or trastuzumab (visit 4). All visits included physical examination, electrocardiogram, blood sampling and CMR. At baseline, visit 3 and visit 4, echocardiograms were also obtained.

3.4. Study safety

Both candesartan and metoprolol have been available for years, and potential side effects are well documented and are usually mild and transitory.

Candesartan is a selective angiotensin II type 1 receptor antagonist widely used in hypertension and heart failure. The most common side effects are headache and dizziness. In addition, candesartan may affect renal function and increase potassium levels.[184]

Metoprolol is a selective β_1 receptor blocker used to treat hypertension, angina pectoris, heart failure and arrhythmias. Metoprolol may cause bradycardia, hypotension, nausea, fatigue, dizziness, depression, and insomnia.[185]

To detect any adverse effects, heart rate, blood pressure and serum creatinine were measured at every study visit, and patients were asked about symptoms. In addition, on routine oncological visits every third week during FEC treatment, safety was monitored by measuring blood pressure and heart rate, as well as creatinine, urea, sodium and potassium. In case of unexpected serious adverse reactions, patients had access to the study-doctor's telephone number. A Data Safety and Monitoring Board with access to the randomization list was responsible should the need of unblinding of therapy arise, and for decisions regarding premature termination of the study. A potentially serious side effect of gadolinium contrast is nephrogenic systemic fibrosis (NSF), a disease that causes thickening of skin and subcutaneous tissues, as well as fibrosis of internal organs. NSF has only been reported in patients with renal failure. Patients with acute renal insufficiency, in dialysis or with glomerular filtration rate (GFR) <30 ml/min are considered at high risk for developing NSF. Patients with GFR between 30 and 59 ml/min have lower risk, and patients with stable GFR above 60 ml/min are not at risk.[186] GFR was measured before each CMR, and if GFR

dropped below 60 ml/ min, or no recent GFR was available, CMR was performed without contrast enhancement.

3.5. Trial registration and ethical approval

Prior to study initiation, the trial was registered in the ClinicalTrials.gov registry (NCT01434134), and the study protocol was approved by the Regional Ethics Committee of South-Eastern Norway (2010/2890).

3.6. Imaging and analysis

3.6.1. Assessment of cardiac function

3.6.1.1. CMR

All CMR examinations included standard b-SSFP cine imaging with one contiguous short axis stack covering the entire ventricles. As these were intended for functional analysis, they were acquired before administration of gadolinium contrast, to ensure optimal contrast between the myocardium and blood. In addition, 3 slices were acquired in each long axis view for visualization of valves, wall movement and for cross reference purposes. To shorten acquisition time, these were acquired after gadolinium injection and before LGE imaging. All image analysis was performed off-line on dedicated, commercially available software (cmr[42]) according to Society for Cardiovascular Magnetic Resonance guidelines.[75] Papillary muscles and trabeculations were included in the ventricular volumes and excluded from LV mass (Figure 4).

3.6.1.2. Echocardiography

Transthoracic echocardiography images were stored offline, and analyzed on dedicated software by Geeta Gulati. Systolic function was assessed by LV global longitudinal peak systolic strain (LV GLS) by speckle tracking in three standard apical views. Diastolic function was assessed by the E/e' and the E/A ratios.

3.6.2. Assessment of cardiac morphology

3.6.2.1. T2 STIR

To assess myocardial edema, conventional short axis breath-hold, black-blood triple inversion recovery T2 images were acquired. A region of interest (ROI) that included as much as possible of the myocardium while avoiding partial volume effects, and one in nearby skeletal muscle was drawn, and the ratio of the signal intensity in the myocardium to that of skeletal muscle was calculated.

3.6.2.2. LGE

LGE imaging was performed starting 10 minutes after injection of 0.2 mmol/kg gadolinium contrast, typically with a 2-dimensional inversion recovery turbo field echo sequence in short axis covering the ventricles, and phase-sensitive 3-dimensional inversion recovery turbo field echo sequences in four chamber and left two chamber axis. LGE volume was assessed semi automatically as areas of more than 5 standard deviations signal intensity above the manually delineated remote myocardium, and the hyperenhanced volume in percent of the myocardium was calculated.

3.6.2.3. T1 mapping and ECV measurements

The T1 mapping sequence was provided free of charge from Philips as a clinical science key. Mid-ventricular, short axis T1 maps were generated offline on cmr[42] from breath-hold, 3(2)3(2)5 MOLLI sequences acquired before and 15 minutes after injection of gadolinium contrast. Typically, 2 or 3 native and post contrast MOLLI sequences were acquired, and after visual assessment of source images for movement and off-resonance artifacts, the sequence of best quality was chosen for map generation. Endo- and epicardial contours were delineated on each T1 map, avoiding adjacent structures to minimize partial volume effects. Areas of LGE were excluded, as were segments with off-resonance artefacts and significant motion artifacts. Native and post contrast blood T1 was obtained by drawing ROIs in the LV cavity, avoiding papillary muscle. Hematocrit was acquired immediately before each CMR examination. ECV fraction, total ECV and total cellular volume were calculated as outlined in the introduction.

3.6.3. Blinding and variability assessment

All CMR evaluations were performed by the author of this thesis, who was blinded for treatment allocation and study order. Fifteen examinations were randomly selected for evaluation of intra-observer variability of both LVEF and T1 measurements. The same samples were evaluated by Florian von Knobelsdorff, at Medical University Berlin, Charité Campus Buch, for assessment of inter-observer variability.

3.7. Biochemical assessment

Baseline blood samples were drawn on inclusion or on the day of baseline examinations, at later time points generally at the same day as CMR examinations. All samples were stored at -80 °C, and were analyzed at the end of the study with the same machine, kit and reagents within a short period of time. Abbott Diagnostics provided reagents for the analysis of cTnI, BNP, galectin-3 and CRP free of charge. Analyses of cTnI, BNP and galectin-3 were performed at the laboratory of the Clinical Research Unit, Division of Medicine, Akershus University Hospital, while the analyses of cTnT and proBNP were performed at the central laboratory of Akershus University Hospital with reagents from Roche Elecsys. Analysis of CRP was performed at Clinic for Medical Diagnostics, Vestre Viken Hospital Trust in Drammen.

3.8. Study end points and statistical analysis

A statistical analysis plan defining a hierarchy of endpoints, as well as time points, statistical analysis strategy and predefined subgroups was finalized before database lock. The primary endpoint of the PRADA trial was change in LVEF from baseline to end of study (EOS), as determined by CMR. The study was designed with power of 0.95 to detect an absolute between-group difference in change in LVEF of 5 ± 5%, which was deemed clinically relevant. After adjusting for potential dropouts, inclusion target was set to 120 patients. Secondary efficacy endpoints included changes in ECV fraction, cTns, LV GLS and LV diastolic function as assessed by E/E'. Tertiary efficacy endpoints included changes in native T1, T2 ratio, LGE, BNP, NT-proBNP, galectin-3, CRP and LV diastolic function as assessed by E/A. Primary and secondary predefined time point for data analyses were EOS, namely the time of completion of planned adjuvant therapy, and completion of FEC, respectively. Subgroup analyses were predefined in patients receiving the highest anthracycline doses.

All efficacy analyses were performed on an intention-to-treat sample (ITT). In addition, per-protocol analyses were performed.

Analyses at primary time point (paper II) were performed by fitting a linear mixed model to each outcome measure from: (1) baseline, (2) after the first cycle of anthracycline therapy, and (3) EOS. When analyzing changes from baseline to end of anthracycline therapy, longitudinal changes and between-groups differences were assessed using paired samples t-tests and independent t-tests for normally distributed data (paper III) and Wilcoxon Signed Rank and Mann-Whitney U tests for non-normally distributed data (paper IV). In addition, in paper IV, multivariate linear regression was used to assess the relationship between cardiac function as assessed by LVEF, LV GLS and E/E', and circulating biomarkers.

4. Summary of Papers

4.1. Paper I

Rationale and Design of the Prevention of Cardiac Dysfunction during Adjuvant Breast Cancer Therapy (PRADA) Trial.

In paper I published early in the trial, we outline the scientific background for initiating the PRADA trial, and describe its design and methodology.

4.2. Paper II

Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 x 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol

The aim of this paper was to test the hypothesis that concomitant therapy with candesartan or metoprolol attenuates the decline in LVEF associated with adjuvant, anthracycline-containing regimens with or without trastuzumab and radiotherapy for early breast cancer

Included were all validly randomized patients who had undergone baseline CMR examination. Six patients were unable to complete CMR due to claustrophobia and were excluded, leaving 120 patients in the ITT population.

Main outcome measures were change in LVEF (primary end point), right ventricular ejection fraction (RVEF), LV GLS, diastolic function (E/E'), and cTnI from baseline to the completion of adjuvant anticancer therapy (EOS).

In the whole population, there was a modest decline in LVEF that was attenuated by candesartan (2.6 % vs. 0.8 %, 0=0.026), but not by metoprolol (1.8 % vs 1.6%, p=0.772). We also observed a decline in RVEF and increase in troponins as further indications of myocardial injury; however, there was no significant impact on the interventions on these outcome measures.

4.3. Paper III

Effect of candesartan and metoprolol on myocardial tissue composition during anthracycline treatment: the PRADA trial

In this paper, we hypothesized that anthracycline treatment was associated with increased ECV fraction and total ECV, and reduced total cellular volume, and that concomitant candesartan or metoprolol treatment could prevent these changes.

All patients who had valid ECV fraction measurements at both baseline and at completion of anthracycline treatment were included. ECV measurements were available from March 2012, and 24 patients underwent baseline examinations before this time. Further 27 patients did not have valid ECV measurements at one or both examination time points, leaving 69 patients in the cohort of this paper.

Main outcome measures were change in ECV fraction, total ECV and total cellular volume from baseline to end of anthracycline containing treatment

There was a significant increase in ECV fraction. Patients who received higher anthracycline doses had a significantly greater increase in ECV fraction and total ECV, as well as a greater decline in LVEF than patients who received lower doses. Patients who received candesartan experienced a significant reduction of total cellular volume, whereas those who did not receive candesartan did not.

4.4. Paper IV

Neurohormonal Blockade and Circulating Cardiovascular Biomarkers During Anthracycline Therapy in Breast Cancer Patients: Results from the PRADA Study

The aim of paper IV was to assess longitudinal change in circulating biomarkers of myocardial injury, dysfunction, inflammation and fibrosis, to assess the effect candesartan and metoprolol on the biomarker response and to assess whether on-treatment changes in biomarker concentrations were associated with decline in left ventricular function.

Included were all validly randomized patients with biomarker measurements at baseline and at completion of anthracycline containing therapy. Five patients did not complete adjuvant treatment as planned or did not provide blood samples at the end of anthracycline therapy, leaving 121 patients in the study cohort.

Outcome measures were change in circulating cTnI and cTnT, BNP, N-terminal pro-BNP, CRP, and galectin-3 from baseline to end of anthracycline containing treatment.

We found that the concentration of all biomarkers increased significantly during anthracycline therapy, and that the increases in cTnI, cTnT and CRP concentration were dose dependent. Metoprolol, but not candesartan attenuated the increases in cTnI and cTnT. None of the changes in biomarker concentrations were associated with change in myocardial function.

5. Discussion

5.1. Methodological considerations

All measurements are subject to error that may affect the validity of research. Random errors are related to sampling variability and affect the precision of the measurement, while systematic errors are deviations from the true value caused by study design or measurement technique and affect the accuracy of the measurement.

5.1.1. Study design

Randomized, controlled trials (RCT) represent the gold standard for determining the effect of an intervention. In the hierarchical ranking of evidence from Oxford Centre for Evidence-Based Medicine, RCTs are at the second highest level, surpassed only by systematic reviews of randomized trials.[187] Randomization of participants minimize allocation bias, and assure a random distribution of confounding factors between groups, and the efficacy of the intervention may be assessed by comparing participants assigned to intervention to the control group.[188,189] A double-blind, placebo controlled design minimizes bias from expectations of effect in both participants and researchers.[190] Blinding of participants may also increase compliance, as they likely are more motivated to adhere to active medication than to inactive placebos. Blinding of researchers is especially important when assessing outcomes, and ideally, assessors should be blinded for treatment allocation and study order.[191] To avoid allocation bias in the PRADA trial, an independent statistician located at Oslo University Hospital performed the randomization. Both participants and study personnel were blinded for treatment allocation throughout the duration of the trial, and all CMR and echocardiography assessments were in addition performed blinded to study order.

A 2 x 2 factorial design is an attractive way to test two hypotheses in one trial with only moderate adjustment of sample size, and allows direct head to head comparison of two interventions. By randomizing patients to two levels of intervention, i.e. 1) candesartan or candesartan placebo and 2) metoprolol or metoprolol placebo, we obtain four intervention groups: 1) candesartan / metoprolol 2) candesartan / metoprolol placebo 3) candesartan placebo / metoprolol and 4) candesartan placebo / metoprolol placebo. When analyzing the effect of candesartan, all patients who received candesartan are compared to all

patients who did not, collapsed across assignment to metoprolol, and vice versa (Table 1).

Table 1 2 x 2 factorial design

	Candesartan	Candesartan placebo	N
Metoprolol	30	32	62
Metoprolol placebo	32	32	64
N	62	64	126

This study design assumes there is no significant interaction between the two interventions, and trials designed to detect the main effect of interventions may be underpowered to statistically detect small interactions. However, when previous studies and physiological reasoning indicate that little interaction is to be expected, a factorial design may be a rational choice.[192,193] We expected little interaction between the interventions, and on testing we found no statistically significant interaction.

5.1.2. Study population

The PRADA participants were a homogenous group of previously generally healthy women with early breast cancer. Patients with previous cardiovascular disease, who are at increased risk of cardiotoxicity, were excluded as they may have clinical indications for one or both study medications, and potential assignment to placebo treatment would be unethical. Also, these patients might have benefitted from treatment with candesartan and metoprolol due to their underlying disease irrespective of adjuvant treatment, thus introducing potential confounders. The anthracycline doses received during the trial were low to moderate, and the cardiotoxic effects and potential benefit of intervention might have been greater in a population with more risk factors and higher doses. However, our cohort is representative of a large number of women with early breast cancer.

5.1.3. CMR systolic function

Ventricular volumes and ejection fraction were calculated from complete stacks of b-SSFP short axis images, recognized as the most accurate and reproducible method. All evaluations were performed by a single assessor, as intra-observer variability tends to be lower than inter-observer variability.[74,75,194,195] Intra-observer variability and inter-observer variability evaluated with an external assessor were very low.

5.1.4. CMR ECV

The T1 mapping sequence initially provided by Phillips was MOLLI 3(2)3(2)5, and this sequence was used without modification for native and contrast enhanced T1 mapping. The MOLLI sequence has been thoroughly evaluated in numerous publications. MOLLI allows highly reproducible quantification of myocardial T1, but is sensitive to extreme heart rates, and prone to slight underestimation of T1.[105,106] Phantom studies have shown that three pause heartbeats lead to less T1 error than two pause heartbeats.[107] However, consistent imaging parameters are essential in longitudinal studies, and small modifications may introduce bias.[108] Therefore, as the initial examinations were done with the 3(2)3(2)5 sequence, we decided to continue this throughout the trial. Also, T1 maps are sensitive to motion,[196] and as we did not have inline motion correction, the shorter scan time of 15 instead of 17 heartbeats may have reduced motion artifacts. Gadolinium significantly shortens T1, and T1 mapping sequences adapted to shorter T1s may improve the accuracy of post contrast T1 measurements,[117] but we used the same scanning parameters for native and post contrast images. These factors, as well as other factors related to protocol, scanner and vendor may affect the accuracy of T1 measurements, i.e. how close the measure is to the true T1 value. However, if tight control is kept of scanning parameters, T1 measurements with the MOLLI sequence are highly reproducible, which may be of greater importance in longitudinal studies.[116] We did not modify the MOLLI sequence during the trial, all examinations were performed on the same scanner, and all T1 maps were generated and assessed with the same cmr[42] release. There is some data that measured ECV fraction increases slightly over time after bolus injection; consequently we carefully timed the contrast enhanced T1 scans to 15 minutes after contrast injection.[117,121] Correction for hematocrit is an important part of the ECV fraction equation, and hematocrit may vary over time. Therefore, hematocrit was measured at the time of examination.

There are several possible approaches to delineating myocardial ROIs. We chose to include the whole myocardium in one short axis slice, excluding segments with significant artifacts. The lateral wall is often thinner than the septum, and more prone to motion artifacts, and placing a ROI in the septum only might have decreased variability.[197,198] However, to minimize bias from partial volume effects and motion, we took care to place the ROI conservatively within the myocardium and excluded segments with significant motion, and intra- and inter-observer agreement for both native and post contrast T1 measurements were excellent.

Both ECV fraction and LV mass assessment by CMR have been extensively validated.[119,199-201] However, the combination of these measurements into the absolute extracellular and cellular volume in mL is a relatively new concept, but is a logical extension, especially in longitudinal studies, as it may provide insight into whether changes in ECV fraction or mass are attributable to interstitial or cellular changes. In a study of patients undergoing aortic valve replacement, Flett et al were able to show that LV hypertrophy regression was cell volume reduction and not regression of fibrosis.[125] Patients undergoing renal denervation for resistant hypertension experienced significant reduction in LV mass without change in the ECV fraction, suggesting a proportionate reduction in myocyte volume and interstitial fibrosis.[124] Conversely, a recent abstract of a small, longitudinal study of Alstrom Syndrome (a genetic, metabolic disease that leads to multiple organ fibrosis) indicated that increasing LV mass was expansion of the extracellular space rather than myocyte hypertrophy.[202]

There are, however, limitations to this approach. Firstly, it makes assumptions of diffuse changes in the heart based on measurements of the extracellular volume in one midventricular slice. While it is likely that anthracyclines and candesartan affect the entire myocardium in equal measures, this is still an approximation. Second, by combining two measures you also combine the uncertainties of both measurements. Finally, there have been no studies documenting the correlation of changes in total cellular and extracellular volumes assessed by CMR to histological changes.

5.1.5. Biomarkers

Standardized blood sample handling and analysis is essential to minimize variability. All blood samples were drawn and handled by experienced study nurses and frozen pending batch analysis. TnI, BNP and galectin-3 analysis were

thawed and analyzed by an experienced research bioengineer at Akershus University Hospital research laboratory. TnT and NT-proBNP analysis were performed at Akershus University Hospital central laboratory, and CRP was analyzed at Clinic for Medical Diagnostics, Vestre Viken Hospital. All analyses were performed over a short period of time, using the same lots, under rigorous quality control. However, random errors related to sample handling may occur. Differences in triggering factors and half-life will influence the dynamic profiles and diagnostic window of the circulating biomarkers, and choice of measurement time point may affect the results.[203] We obtained blood samples at baseline and on average about 14 days after completion of anthracycline and additional therapy and may have missed the optimal diagnostic window of one or more biomarkers. Adding measurement time points would have supplied information on the dynamic profile of each biomarker. However, for ethical and practical reasons, we did not want to impose additional hospital visits to an already demanding participant schedule.

5.1.6. Echocardiography

Accuracy of echocardiographic indices are influenced by image quality and operator experience. All patients in our cohort had recently undergone breast cancer surgery, and in some patients postoperative wound or silicone implants impaired the acoustic window, and especially speckle-tracking is sensitive to acoustic shadowing. In fact, only 82 and 83 patients had valid strain measurements at both baseline and after anthracyclines, and baseline and end-of study, respectively, thereby reducing the statistical power to detect differences with this method. Diastolic function was assessable in most patients. However this measure is sensitive to loading conditions, which in our cohort may be influenced by cancer therapy related nausea and vomiting, as well as by effects of metoprolol on heart rate and ventricular filling. Vendor differences may also affect results, therefore all image acquisitions were made with single vendor ultrasound scanners, and all off-line analyses were performed with the same software.

5.1.7. End points

A surrogate endpoint is a measurement that substitutes a clinically meaningful endpoint when comparing treatments in clinical trials, and may reduce sample size and trial duration. As clinical heart failure due to contemporary adjuvant therapy is relatively infrequent and may become apparent years after exposure,

we relied on surrogate endpoints to test the effect of candesartan and metoprolol to prevent cardiac dysfunction during adjuvant breast cancer therapy. For a surrogate endpoint to be valid there should be extensive evidence that there is a proportionate relationship between the surrogate endpoint and outcomes, and that changes induced by intervention can be expected to predict changes in clinically meaningful endpoints, in a variety of populations.[204,205] We chose change in LVEF as the primary endpoint as it has been shown to be a powerful predictor of survival in numerous populations, including heart failure patients, and because favorable effects on LVEF by ACEIs and beta-blockers have been associated with improved clinical outcomes. In a recent study of 2625 patients receiving anthracyclines, end-chemotherapeutic LVEF was shown to be independently associated with occurrence of cardiotoxicity.[206] Also, studies have shown a proportionate relationship between LVEF and change in LVEF and mortality.[204,207-211]

Secondary and tertiary endpoints in this thesis include established and novel biomarkers. Incremental increases of ECV fraction have been associated with morbidity and mortality in cross-sectional studies,[212-214] and although there is optimism that ECV fraction, total extracellular and cellular volume will prove important biomarkers and surrogate endpoints in longitudinal studies, especially in interventional trials, evidence is still pending.[108,120,122-125,202,215] The prognostic value of cTns and natriuretic peptides has been documented in a number of clinical settings, including during high dose anthracycline therapy, and there is proportionality between biomarker elevation and adverse outcomes.[15,129,131,132,137,138,216-218] CRP is associated with increased risk of heart failure and mortality [143,219] and has been shown to increase during anthracycline and trastuzumab therapy, but whether this increase may predict cardiotoxicity is unclear.[134,140] Galectin-3 is also a predictor of heart failure,[146] but to what extent is will be a useful biomarker during adjuvant therapy is largely unexplored.[134,141,147]

The predefined primary time point for data analyses of study end-points was end of adjuvant therapy. The rationale for this was that women with early breast cancer receive multiple hits to the heart during adjuvant therapy, and to assess the effect of neuroendocrine blockade in this large patient group, inclusion of the whole course of potentially cardiotoxic treatment seemed reasonable.[220] However, anthracyclines, radiotherapy and trastuzumab exert their harmful effects on the myocardium in different ways, and while all patients received anthracyclines over

the course of 10-12 weeks, treatment received after anthracyclines and time to end of study varied. Therefore, we predefined end of anthracycline therapy as a secondary time point for data analyses, facilitating analysis of the effect of anthracyclines, irrespective of later exposure to other cardiotoxic therapies.

5.1.8. Sample size

The decline in LVEF in the placebo group was less than anticipated, and a smaller between-group difference reduces the statistical power to reject the null hypothesis.[221] We may thus have been underpowered to detect a small effect of metoprolol. Also, as sample size calculations were based on the primary endpoint, the statistical power for analysis of some of the secondary endpoints and subgroups may have been limited.

5.2. Discussion of results

5.2.1. Detection of cardiotoxicity

Patients in the PRADA trial all received contemporary adjuvant treatment for early breast cancer, with regimens striving for an optimal tradeoff between anticancer effect and risk of cardiotoxicity. Still, subclinical signs of cardiotoxicity were detectable by CMR and circulating biomarkers. We showed that patients who did not receive candesartan experienced a modest decline in LVEF as assessed by CMR during the complete course of adjuvant therapy (paper II), as well as during anthracycline therapy (paper III). We also showed that patients who received higher anthracycline doses had a greater fall in LVEF than patients who received lower doses (paper III). In addition we found dose-dependent increases in ECV fraction and total ECV by CMR (paper III) as well as in circulating biomarkers of myocardial injury and inflammation (paper IV) during anthracycline treatment. The dose dependency supports the notion that these are true signals of cardiotoxicity,[222] and the findings are in line with previous evidence that contemporary treatment regimens with low dose anthracyclines, radiation therapy and trastuzumab may harm the heart.[4] The magnitude of change was not large and none of the women in our study experienced clinical heart failure. However, the decline of 2.6 percent points in patients who did not receive candesartan in this previously generally healthy population is in accordance with the decline of 2-5 percentage points reported in recent studies, in part conducted on cohorts at higher risk.[99,101,183,206,223,224] Similarly, the increase in morphological CMR indices was small, and well within the normal range, indicating that adjuvant therapy for early breast cancer is relatively safe in the short term.

Recently, in a research letter in JACC Cardiovascular Imaging,[225] Meléndez et al showed that in patients receiving anthracycline doses slightly higher than the highest doses in our study, ECV fraction increased with 1.7 percent points from baseline to 3 months after anthracycline initiation, compared to 3.4 percent points in the higher dose group in the PRADA trial. Meléndez did not report on LV mass or total ECV, but inferred that the increase was due to expansion of the extracellular space by edema or fibrosis, although in theory it could be caused by reduced LV mass and total cellular volume. In our trial, by taking into account changes in myocardial mass, we registered that even though lower anthracycline doses were associated with a slight elevation of ECV fraction, this was explained

by reduced cellular volume, and only the highest anthracycline dose was associated with expansion of the extracellular space. (Figure 3, paper III)

The extracellular space may be expanded by edema or fibrosis. In a recent animal study, Farhad et al [226] showed that mice exposed to 5 weeks of high anthracycline doses displayed increased T2 values at cessation of chemotherapy, and increased ECV fraction 5 weeks after end of chemotherapy. The increased T2 values and ECV fraction correlated strongly with increased water content and histological findings of fibrosis, respectively. This is in line with previous data that inflammation plays a role in anthracycline-induced cardiotoxicity, and that cardiomyocyte injury occurs early. Later, histopathologic signs of cell damage disappear, indicating that damaged cells either have been repaired, or lost followed by replacement fibrosis.[227,228] Although edema may expand the myocardial extracellular space, and previous human studies indicate that myocardial edema in conditions such as acute myocarditis and systemic capillary leak increases ECV fraction,[229-231] the increased water content at week 5 was not associated with increased ECV fraction in Farhads data. These findings in mice treated with high anthracycline doses and examined with a 9.4 T scanner may not be directly transferrable to human studies. However, the discrepancy highlights the point made by Schelbert and Messroghli in a comprehensive review in Radiology in 2016 that there is still not enough data to determine the value of ECV fraction in inflammatory myocardial diseases.[123] The lack of numerically significant increase in T2 ratio from baseline to end of anthracyclines compared to for instance values reported in acute myocarditis [232] may suggest that any edematous component in our cohort one to two weeks after completion of anthracycline therapy is minor. There are, however some inherent challenges in assessing myocardial edema with T2 ratio. Long acquisition time and relatively low signal-to noise ratio may reduce image quality, and as it is calculated as the ratio of signal intensity of the myocardium to that of skeletal muscle, simultaneous affection of skeletal muscle may attenuate increases in T2 ratio.[91] T2 mapping overcome some of these limitations, [233] and in Meléndez' cohort, septal T2 time was slightly elevated three months after initiation of anthracycline therapy.[225] T2 maps were not acquired in the PRADA trial, thus we cannot ascertain to what extent the increase in total ECV was caused by edema or fibrosis, or a combination.

Although studies have shown increased native T1 in fibrosis and edema,[115] results from studies on anthracycline cardiotoxicity are sparse. While Meléndez [225]

found a small, but significant increase in native T1 in patients at three months, native T1 was increased during the early, edematous phase of high-dose anthracycline therapy in mice, but not during subsequent fibrosis.[226] Native T1 did not increase during anthracycline therapy in our data. This may be because native T1 mapping measures the signal from both the extracellular and the intracellular space, and, the different effects of candesartan and anthracyclines on these compartments may attenuate the differences. Also, the numerical T1 differences in diffuse fibrosis may be small, and there is overlap between patients groups.[115]

Anthracycline treatment was not associated with reduced LV mass and total cellular volume. Results from previous studies on the effect of anthracycline therapy on LV mass are conflicting. A retrospective, observational study of 91 patients with clinically diagnosed anthracycline cardiotoxicity reported an inverse association between anthracycline dose and LV mass, as evaluated by CMR.[234] A study of 115 children with previous anthracycline exposure longitudinally assessed by echocardiography showed progressive loss of LV mass relative to body surface area 6 to 9 years after treatment.[235] On the other hand, a study of 62 survivors of childhood cancer did not find that LV mass as assessed by CMR differed from normal values.[236] These populations differ significantly from ours of previously healthy adult women. The first study included patients with suspected cardiotoxicity and confirmed depressed LVEF after anthracycline therapy, who likely had significant myocardial damage. The two other studies observed children, who may be more susceptible to anthracycline cardiotoxicity.[235,237] Our findings indicate that contemporary anthracycline doses in adults do not induce significant short-term myocardial loss.

We found a dose-dependent increase in cTns and CRP as well as an increase in galectin-3 concentrations (paper IV) during anthracycline therapy, supporting the concept that anthracyclines induce cell damage, inflammation and fibrosis.[127,128,134,141,238] Troponin levels in the whole cohort were lower at the end of adjuvant therapy than at after anthracycline therapy only, but were elevated compared to baseline levels (paper II). Metoprolol treatment has been shown to increase natriuretic peptides,[239,240] and BNP and NT-proBNP increased only in patients treated with metoprolol, likely due to hemodynamic effects of the drug, and not cardiac dysfunction (paper II and IV).

While several studies have shown that elevated cTns during high dose chemotherapy predict subsequent cardiotoxicity,[15,132,241] the association is less

clear during treatment with low to moderate anthracycline doses.[127,128,134,135,242-245] Also, several studies have shown that increases in natriuretic peptides may predict subsequent cardiotoxicity from chemotherapy,[138,243,244] but the results are not consistent.[242,246] There may be several reasons for these discrepancies. These studies include various cancer entities, treatment regimens and cohorts with different risk factors for cardiotoxicity. Some studies evaluate change in LVEF as assessed by echocardiography [15,132,241,242,244] others by radionuclide imaging.[243,246] Endpoints include cardiotoxicity as a dichotomous outcome, with different definitions or cardiotoxicity,[15,242,243] and association between biomarker change and change in LVEF.[132,241,243,244,246] Also, differences in biomarker sampling schemes may affect the results. We did not find that early changes in circulating biomarkers after anthracycline therapy correlated significantly with decline in cardiac function. Firstly, it may be that the observation period is too short, as deterioration of cardiac function may develop over time.[4,35,206] Secondly, many of the previous trials have been conducted with higher anthracycline doses in patients with more risk factors,[15,134,243-245] and it may be that the cardiotoxic insult of low to moderate anthracycline doses in our healthy population is too small to inflict clinically significant myocardial damage. Thirdly, as discussed under methodological considerations, it may be that different timing or additional measurements would have been more predictive. Finally, the possibility of some degree of publication bias must be considered.

The dichotomous concept of early and late anthracycline cardiotoxicity has recently been challenged, and there is reason to believe there is a continuum from cardiotoxic damage at the time of exposure through subclinical cardiac dysfunction to clinical symptoms of heart failure, and the incidence of cardiac dysfunction increases over time. In the prospective study of 2625 anthracycline receiving patients, participants were monitored with LVEF measurements every three months during and for the first year after anthracycline containing therapy, then every 6th month for four years and then yearly.[206] This study showed that median time to develop cardiotoxicity was 3.5 months after end of therapy and that 98 % percent of the cardiotoxicities were detected within the first year after therapy completion. The strongest predictors of cardiotoxicity were end of chemotherapy LVEF and anthracycline dose. Thus, in the upcoming one to two year assessment of the PRADA population it will be interesting to see whether there is a further deterioration of cardiac function, and whether the early indices of cardiotoxicity predict this decline.

5.2.2. Prevention of cardiotoxicity

While the initial cardiotoxic insult exert direct damage to the cardiomyocytes, compensatory cardiac remodeling may eventually become maladaptive, and cause further deterioration of cardiac function. Thus, preventive strategies should ideally aim to minimize myocyte damage as well as maladaptive remodeling processes.[227] In the PRADA trial we found that candesartan and metoprolol exerted different protective effects.

5.2.2.1. Myocardial damage

Troponins are sensitive biomarkers of myocardial damage, and there are ample studies that show that troponins increase during anthracycline therapy. Trastuzumab treatment is usually only associated with troponin increase when given concomitantly with or after anthracyclines, and this is likely due to ongoing myocardial damage due to anthracycline therapy as well as trastuzumab mediated inhibition of repair mechanisms in cardiomyocytes damaged by anthracyclines.[133,141,247] Left sided radiotherapy for breast cancer has also been associated with troponin increase, although its value as a biomarker of radiotherapy-induced myocardial damage has been less investigated.[127,248] Our finding of less troponin increase in the metoprolol group suggests that metoprolol attenuates cardiomyocyte damage during anthracycline treatment. In animal models, stimulation of β1 adrenoceptors increases apoptosis, and selective β1 receptor blockade abolishes this effect.[249,250] Metoprolol is a selective β1 receptor blocker, and may exert its protective effect through inhibition of pro-apoptotic pathways. Reduced wall stress could theoretically also contribute in protection against apoptosis, however, as we did not find that candesartan attenuated cTn increase, despite similar blood pressure reductions, this is unlikely the only explanation.[227,251] We found no impact of the intervention on troponin level increase from baseline to end of adjuvant treatment, likely because radiotherapy and trastuzumab did not cause significant additional myocardial destruction, as troponin levels were lower at the end of adjuvant therapy than at the end of anthracycline therapy alone.

5.2.2.2. Cardiac remodeling

Although candesartan did not prevent myocyte damage, as assessed by troponin release, it was associated with preservation of LV systolic function during adjuvant therapy (paper II and III) as well as a significant reduction of total

cellular volume (paper III). Candesartan and metoprolol had similar effects on blood pressure, but only candesartan prevented the small decline in systolic function, hence it is likely that other effects than decreased afterload contributed. Activation of the RAAS plays an important role in the pathogenesis of heart failure and adverse cardiac remodeling,[162] and has been implicated as a contributing factor in the development of anthracycline cardiotoxicity.[169-172] In an animal study from 2011, the researchers showed that administration of the anthracycline doxorubicin to rats reduced ventricular contractility, impaired mitochondrial function and increased apoptosis.[252] Concomitant administration of the ACEI enalapril significantly attenuated the decline in systolic function, did not prevent apoptosis but prevented impairment of mitochondrial function. It is possible similar mechanisms could explain why candesartan preserved systolic function, despite having no significant effect on myocardial injury as assessed by circulating troponins in our study.

Angiotensin II induces myocyte hypertrophy independently of pressure overload [161,253], and angiotensin receptor blockade has been shown to reduce LV mass in hypertensive patients.[254,255] In the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) Study, treatment of hypertensive patients with ECG signs of LV hypertrophy with the ARB losartan was associated with significantly greater decline in LV mass than treatment with the beta-blocker atenolol, despite similar reductions in blood pressure.[254] In our study of normotensive patients, candesartan but not metoprolol or anthracycline dose was associated with reduced total cellular volume, and candesartan treatment did not augment troponin increase. This supports the hypothesis that angiotensin receptor blockade attenuates angiotensin IIs growth promoting effects on the myocardium. Also, candesartan's preservation of LV systolic function during anthracycline therapy indicates that this is not associated with impaired myocardial function.

Although beta-blockade has been proven efficient in treatment of heart failure,[178] and there are studies that suggest that beta-blockade during anthracycline therapy may be beneficial,[180-183] we did not find that metoprolol attenuated the decline in systolic function from baseline to EOS or to end of anthracycline therapy. There may be several reasons for this. Firstly, beta-blockade counteract the deleterious effects of sympathetic activation in heart failure,[178] and it may be the benefits of beta-blockade does not apply in the absence of heart failure. Secondly, several of the studies on beta-blockade during anthracycline therapy have used carvediolol,[181-183] a third generation, non-selective beta-blocker with additional

alpha blocking and potent antioxidant effects. It may be that carvedilol, through reduction of oxidative stress, had been more effective.[256] Finally, as the decline in LVEF was less than originally anticipated, the study may be underpowered to rule out a beneficial effect.

5.2.3. Subsequent studies

Recently, two papers have been published on cardio-protection during trastuzumab therapy for breast cancer. In the MANTICORE trial, which included 99 patients, intervention with the beta-blocker bisoprolol, and the ACE inhibitor perindopril, did not protect against change in LV end diastolic volume, the predefined primary outcome. However, bisoprolol, and to a lesser extent perindopril attenuated early decline in LVEF, a secondary endpoint.[257] In a Dutch study of 210 patients, Boekhout et al found that candesartan did not prevent occurrence of cardiac events.[258] These studies differ in significant aspects from the PRADA trial. First and foremost, both trials studied the effect of cardio-protection during trastuzumab therapy, whereas the PRADA trial assessed cardio-protection during the whole course of adjuvant therapy for early breast cancer, including anthracycline therapy, trastuzumab and radiotherapy. While the MANTICORE trial mainly included patients without previous anthracycline exposure, all patients in Boekhout's data had recently received anthracyclines, and intervention and examinations commenced after this had been completed. The MANTICORE trial, like the PRADA trial used CMR to assess end-diastolic volume and LVEF. Boekhout et al's primary endpoint was occurrence of cardiac events defined as decline in LVEF of>15% or an absolute value<45%, where LVEF was assessed by the less sensitive methods echocardiography or MUGA. The inconsistent results may be explained by differences in exposure to anthracyclines, choice of end-points and sensitivity in the methods of measuring cardiac function.

6. Limitations

The PRADA study cohort consisted of previously relatively healthy women scheduled for contemporary adjuvant treatment with low to moderate anthracycline doses with or without trastuzumab and radiotherapy for early breast cancer. These patients were at relatively low risk of cardiovascular complications, and the measured changes in indices of cardiotoxicity were modest. Patients with more risk factors treated with higher anthracycline doses might have benefitted

more from preventive therapy, but these patients might have had a clear indication for intervention with angiotensin antagonists and/or beta-blockers. By design, the study was not powered to detect differences in clinical heart failure or other cardiac events. The decline in systolic function was generally minor and the difference between groups less than *a priori* defined as clinically important. Not all participants in the PRADA trial had ECV fraction measurements. Finally, the observation period did not extend beyond the duration of adjuvant therapy, and signs of cardiotoxicity may appear months after end of therapy. However, long-term follow up of the PRADA cohort is ongoing.

7. Future Perspectives

Beta-adrenergic and angiotensin receptor blockade exerted different beneficial effects, and it may be reasoned that combination therapy is a promising approach in the effort to prevent cardiac dysfunction from adjuvant therapy. However, before conclusions can be drawn about implementing preventive therapy, further research must demonstrate clinically meaningful benefits. The PRADA cohort has completed their one to two year follow-up, and assessment of the examinations is underway. Larger studies including patients at higher risk will be important. Since completion of the PRADA trial, the guidelines of the Norwegian breast cancer group's for systemic adjuvant treatment have been revised, and all patients scheduled for anthracycline treatment will now receive four cycles of 90 mg/m² epirubicin. Thus, peak and cumulative anthracycline doses will be close to the doses associated with the most signs of cardiotoxicity in the PRADA trial, and these patients should be considered for future trials. Also, there is a need for trials in other populations with different cancer entities and treatment combinations, as well as cohorts including men. In the era of personalized medicine, early risk stratification and identification of patients who will benefit the most from preventive measures will gain importance. Thus, sensitive imaging and circulating biomarkers such as those used in the PRADA trial as will be valuable tools in future trials.

8. Conclusions

Adjuvant therapy for early breast cancer is associated with small changes in both cardiac function and myocardial composition, as assessed by CMR, and increase in circulating biomarkers of myocardial injury, inflammation and fibrosis. Early changes in biomarkers are not associated with short-term change in systolic function, but still, a panel of CMR indices and circulating biomarkers are potentially valuable tools in longitudinal studies on cardiotoxicity.

Treatment with candesartan, but not metoprolol has a beneficial effect on cardiac remodeling, as assessed by preservation of systolic function and a small anti-hypertrophic effect. Metoprolol, but not candesartan attenuates myocardial injury, as assessed by circulating troponins during anthracycline therapy.

Long term follow-up and data from other trials, especially in higher risk cohorts is needed to determine whether prophylactic angiotensin and beta-adrenergic receptor blockade during adjuvant breast cancer therapy should be implemented.

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Papers

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II

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AHA FASTTRACK
CLINICAL RESEARCH

Heart failure/cardiomyopathy

Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 × 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol

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Aims Contemporary adjuvant treatment for early breast cancer is associated with improved survival but at the cost of increased risk of cardiotoxicity and cardiac dysfunction. We tested the hypothesis that concomitant therapy with the angiotensin receptor blocker candesartan or the β-blocker metoprolol will alleviate the decline in left ventricular ejection fraction (LVEF) associated with adjuvant, anthracycline-containing regimens with or without trastuzumab and radiation.

Methods and results In a 2 × 2 factorial, randomized, placebo-controlled, double-blind trial, we assigned 130 adult women with early breast cancer and no serious co-morbidity to the angiotensin receptor blocker candesartan cilexetil, the β-blocker metoprolol succinate, or matching placebos in parallel with adjuvant anticancer therapy. The primary outcome measure was change in LVEF by cardiac magnetic resonance imaging. *A priori*, a change of 5 percentage points was considered clinically important. There was no interaction between candesartan and metoprolol treatments ($P = 0.530$). The overall decline in LVEF was 2.6 (95% CI 1.5, 3.8) percentage points in the placebo group and 0.8 (95% CI −0.4, 1.9) in the candesartan group in the intention-to-treat analysis (*P*-value for between-group difference: 0.026). No effect of metoprolol on the overall decline in LVEF was observed.

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The work was performed at Akershus University Hospital.

Conclusion In patients treated for early breast cancer with adjuvant anthracycline-containing regimens with or without trastuzumab and radiation, concomitant treatment with candesartan provides protection against early decline in global left ventricular function.

Keywords Angiotensin antagonist • β-Blocker • Breast cancer • Cardiomyopathy • Imaging • Biomarkers

Introduction

Progress in detection and treatment of breast cancer during the past two decades has led to substantial improvement in life expectancy but at the cost of increased risk of unintended side effects of cancer therapy.[1] Adjuvant breast cancer treatment may encompass anthracycline-containing chemotherapy and in patients with more aggressive human epidermal growth factor receptor-2 (HER-2)-positive cancers, the use of higher doses of anthracyclines followed by taxanes and the anti-HER-2 agent trastuzumab. Both anthracyclines and trastuzumab have been associated with cardiotoxicity and increased risk of developing asymptomatic and symptomatic cardiac dysfunction.[2–7] Given the increasing number of long-term survivors after breast cancer treatment, cardiotoxicity has been recognized as a major concern in oncology.[1]

Neuroendocrine blockade, including treatment with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and β-blockers, has proved effective in reducing mortality and morbidity in all stages of heart failure, and to prevent the transition from asymptomatic to symptomatic left ventricular dysfunction.[8–10] Experimental studies in animals[11] as well as observational studies[12] and small-scale, randomized, open-label,[13,14] single-blind,[15,16] or double-blind,[17] clinical trials in heterogeneous patient populations with different cancer types and treatment regimens have suggested a potential benefit from early initiation of angiotensin-converting enzyme inhibitors and β-blockers in preventing anthracycline-induced left ventricular dysfunction.[12–18] However, a very recent meta-analysis identified only 79 breast cancer patients who had previously been included in randomized studies of β-blockers and 47 patients who had been included in randomized studies of angiotensin-converting enzyme inhibitors or receptor blockers,[18] and currently no data are available from randomized, placebo-controlled, double-blind trials in breast cancer patients assessed with cardiac magnetic resonance imaging (MRI) and highly sensitive biochemical markers of cardiac injury. We therefore conducted a randomized, 2 × 2 factorial, placebo-controlled, double-blind clinical trial to test the hypotheses that concomitant therapy with the angiotensin receptor blocker candesartan or the β-blocker metoprolol will attenuate the decline in left ventricular ejection fraction (LVEF) associated with adjuvant, anthracycline-containing regimens with or without trastuzumab and radiation for early breast cancer.

Methods

Study design and participants

PRevention of cArdiac Dysfunction during Adjuvant breast cancer therapy (PRADA) was a 2 × 2 factorial, randomized, placebo-controlled, double-blind clinical trial conducted at Akershus University Hospital, Norway. The study complied with the Declaration of Helsinki. The study protocol was approved by the Regional Ethics Committee of South-Eastern Norway (2010/2890), and the trial was registered in the ClinicalTrials.gov registry (NCT01434134) prior to study initiation. All participants provided written, informed consent.

The rationale for and design of the study have been described in detail previously.[19] In brief, women who after breast cancer surgery in the period between September 2011 and September 2014 were scheduled to initiate adjuvant chemotherapy with 5-fluorouracil, epirubicin, and cyclophosphamide (FEC) and had no serious concomitant illness, prior cardiovascular disease, and indication or contraindications for the study drugs were eligible for inclusion. Detailed study inclusion and exclusion criteria are listed in Supplementary material online, *Table S1*.

Randomization and masking

Participants were randomly assigned on a 1:1:1:1 basis to receive one of the following treatment combinations: candesartan cilexetil 32 mg q.d. and metoprolol succinate 100 mg q.d.; candesartan cilexetil 32 mg q.d. and placebo q.d.; metoprolol succinate 100 mg q.d. and placebo q.d.; or placebo and placebo q.d. Details on patient inclusion and randomization are described in the Supplementary material online. *Figure 1* summarizes patient screening and randomization. A similar figure for the per-protocol cohort is provided in the Supplementary material online, *Figure S1*.

Procedures

Patients were examined serially with cardiac MRI, blood samples, physical examinations, and electrocardiograms at the following time points during the trial: at baseline, after completion of the first cycle of anthracycline therapy, after completion of the final cycle of anthracycline therapy, and for those concerned, at completion of trastuzumab or radiation therapy (Supplementary material online, *Figure S2*). Echocardiography was performed at the same time points, except for after completion of the first cycle of anthracyclines. The duration of adjuvant therapy ranged from 10 to 61 weeks depending on the anticancer regimens (Supplementary material online, *Figure S2*).

Initiation of intervention commenced after baseline examination and prior to initiation of chemotherapy. Dose titration is described in detail in the Supplementary material online. Starting dose for candesartan cilexetil was 8 mg and for metoprolol succinate 50 mg, target dose 32 and 100 mg, respectively. Compliance was registered by counting residual tablets on every second visit during FEC treatment and every third visit during trastuzumab treatment. In addition, the patients were given a diary to register intake of tablets.

All cardiac MRI examinations were performed on a 1.5-T MRI scanner (Achieva; Philips Medical Systems, Best, The Netherlands), using a five-element phased-array cardiac coil. Breath-hold, steady-state-free-precession sequences in contiguous, 8 mm thick short-axis images covering the entire ventricles were used to quantify ejection fraction. All image analyses were performed according to Society for Cardiovascular Magnetic Resonance guidelines[20] by a single, board-certified radiologist (S.L.H.) blinded for treatment allocation and study order.

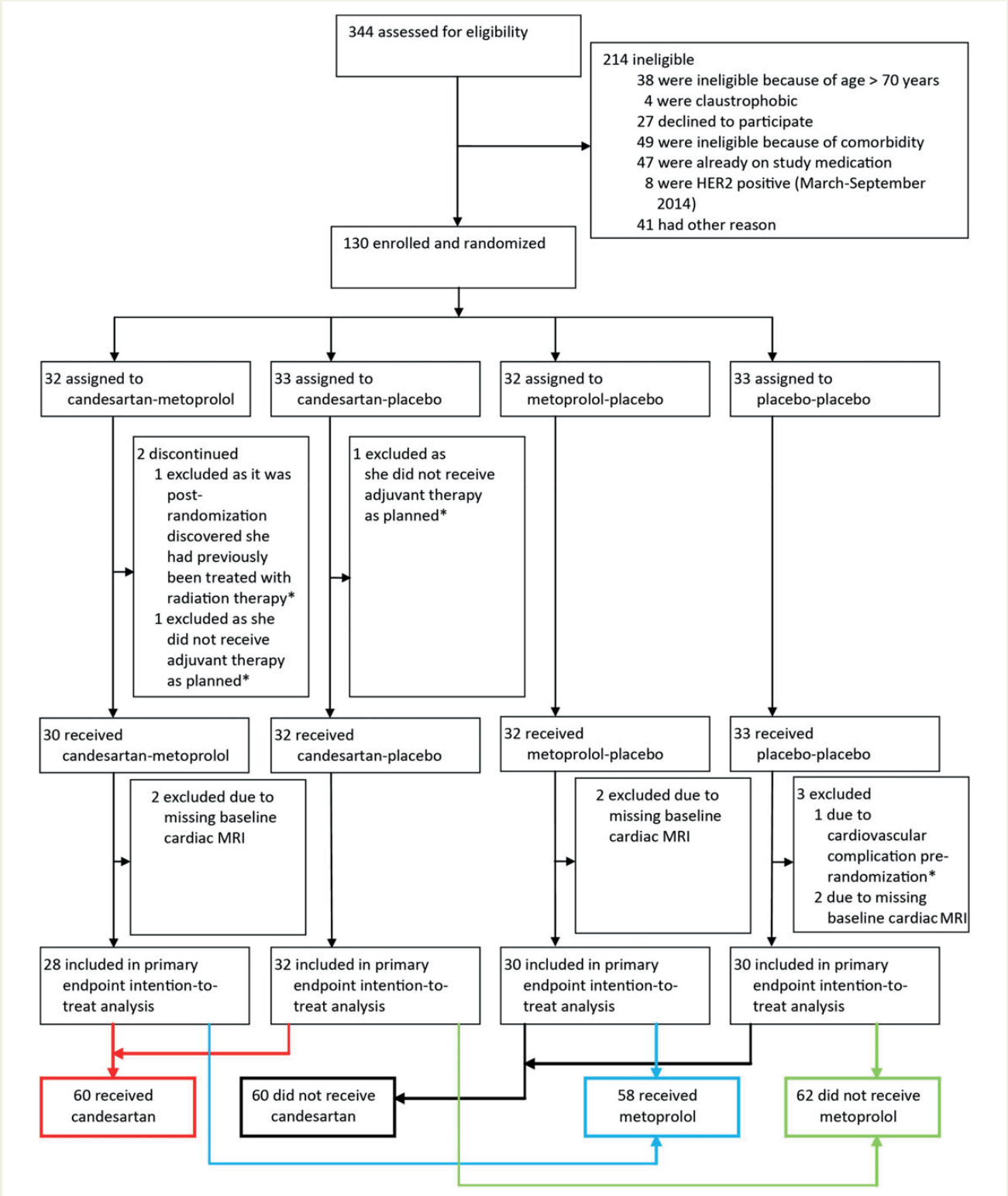

Figure 1 Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): screening and randomization. *Excluded from all analysis. The intention-to-treat population included all patients who had a valid measurement for the primary outcome, received chemotherapy, and had no pre-randomization cardiac complications. HER, human epidermal growth factor receptor; MRI, magnetic resonance imaging.

Transthoracic echocardiography was performed by using a Vivid E9 (GE Vingmed, Horten, Norway). Images were digitally stored for offline analysis on custom software (EchoPAC, GE Vingmed, Horten, Norway). Left ventricular, two-dimensional peak systolic global longitudinal strain was analysed by an offline semi-automated speckle tracking imaging technique from the three standard apical views. Diastolic function was assessed by the ratio between peak early (E) transmitral velocity by pulsed Doppler and peak early tissue Doppler (E′) by averaging septal and lateral E′ at the base of septal and mitral leaflet, respectively. Analyses were performed by a board-certified physician (G.G.), who was blinded to treatment assignment and study order. Detailed descriptions of the cardiac MRI and echocardiographic analyses are provided in the Supplementary material online.

Cardiac troponin I in serum was measured by using an assay from Abbott Diagnostics: ARCHITECT STAT High Sensitive Troponin, as described previously.[21] The level of detection for this assay has been reported to be 1.2 ng/L (range 0–50 000 ng/L) and the level of blank 0.8 ng/L.[22] Samples with a level below or equal to the level of blank (i.e. 0.8 ng/L) were assigned a value of 0.8, whereas levels below or equal to the level of detection (i.e. 1.2 ng/L) and greater than the level of blank, were assigned a value of 1.2 ng/L. The coefficient of variation of 10% has been observed at a concentration of 3.0 ng/L. B-type natriuretic peptide (BNP) in plasma was measured by a chemiluminescent microparticle immunoassay (BNP, Abbott Diagnostics; ARCHITECT). The level of detection is 10 pg/mL. Samples with a level <10 pg/mL were assigned a concentration of 5 pg/mL.

Outcome measures

The primary outcome measure of the trial was change in LVEF from baseline to the completion of adjuvant anticancer therapy, as determined by cardiac MRI. Secondary outcome measures included change in right ventricular ejection fraction, as determined by MRI, left ventricular peak systolic global longitudinal strain by two-dimensional speckle tracking imaging, diastolic function (E/E′), and concentrations of cardiac troponin I by a high-sensitivity assay. Other biomarker and echocardiographic indices of diastolic function were considered tertiary outcome measures. A Data Safety and Monitoring Board consisting of a cardiologist, an oncologist, and a statistician was constituted prior to the initiation of the study and monitored adverse events.

Statistical analysis

With α of 0.05, and power $(1 - \beta)$ of 0.95, 26 patients treated with candesartan and 26 patients treated with metoprolol were required to detect an absolute between-group difference in change in LVEF of $5 \pm 5\%$ (SD) percentage points. With a dropout rate of 17%, the adjusted targeted inclusion was estimated to be a minimum of 120 patients. Out of the 120 patients included in the analysis, 28 received candesartan–metoprolol, 32 candesartan–placebo, 30 metoprolol–placebo, and 30 placebo–placebo (*Figure 1*).

The primary efficacy analysis was performed on an intention-to-treat sample consisting of all validly randomized patients with at least baseline MRI, and a per-protocol sample. All secondary efficacy analyses were also performed on both the intention-to-treat sample and a per-protocol sample. The per-protocol analysis excluded patients who did not have baseline and end-of-study MRI measurements, were not compliant to intervention or discontinued their study medication, withdrew consent, or did not complete adjuvant therapy.

For each continuous efficacy endpoint, we fitted a linear mixed model to all available measurements from three time points: (i) baseline, (ii) after completion of the first cycle of anthracycline therapy, and (iii) end-of-study (either after completion of the final cycle of anthracycline therapy or the completion of trastuzumab or radiation therapy). All models included fixed effects for time, candesartan treatment, metoprolol treatment, candesartan treatment × time interaction, metoprolol treatment × time interaction, age, and left-sided radiation, and a random intercept. To investigate possible interactions between the two treatments, we fitted additional models that included a candesartan × metoprolol interaction term, and applied a likelihood ratio test to the models with and without the treatment interaction term. No statistically significant treatment interactions were observed. Based on the fitted models without the treatment interaction term, we estimated baseline, end-of-study (i.e. the final visit), and change from baseline to end-of-study mean values (with 95% CI) for patients in four groups: (i) treated with candesartan, (ii) not treated with candesartan, (iii) treated with metoprolol, and (iv) not treated with metoprolol. The treatment effects were estimated as the between-group difference in change from baseline to end-of-study for the comparisons of candesartan vs. no candesartan and metoprolol vs. no metoprolol. Troponin I values were log transformed before inclusion in the linear mixed models. All terms in the linear mixed models were pre-specified in the statistical analysis plan.

A *P*-value of <0.05 was considered statistically significant. The reported *P*-values are two-sided and not adjusted for multiple comparisons. The statistical analyses were carried out with Stata 14.0 (StataCorp LP).

Results

Between September 2011 and September 2014, 120 patients with early breast cancer having surgery at Department of Surgery at Akershus University Hospital and scheduled for adjuvant therapy with the anthracycline epirubicin were enrolled in the trial and validly randomized to one of the four treatment groups (*Figure 1*). The four groups were well-balanced concerning patient characteristics at baseline and planned adjuvant anticancer therapy (*Table 1*). Details of the cancer characteristics are given in the Supplementary material online, *Table S2*. Adjuvant therapy was administered according to the recommendations of the Norwegian Breast Cancer Group. All patients received FEC, and if indicated taxanes ($n = 100$; 79.4%), trastuzumab ($n = 28$; 22.2%) and radiotherapy ($n = 82$; 65.1%). No patient developed symptomatic heart failure during the study period.

There was no statistical interaction between candesartan and metoprolol treatment on the primary endpoint ($P = 0.53$) or on any of the secondary endpoints. Accordingly, the patients in the two groups receiving candesartan were compared with patients receiving placebo–placebo or metoprolol–placebo (*Table 2*). The overall decline in the primary outcome measure from baseline to the end-of-study was 2.6 (95% CI 1.5, 3.8) percentage points in the placebo group and 0.8 (95% CI −0.4, 1.9) percentage points in the candesartan group in the intention-to-treat analysis (*P*-value for between-group difference in linear mixed model analysis: 0.026). Corresponding values in the per-protocol analysis were 2.6 (95% CI 1.4, 3.8) percentage points in the placebo group and 0.6 (95% CI −0.6, 1.8) percentage points in the candesartan group ($P = 0.021$ in mixed linear model). Notably, the effect of candesartan on change in LVEF was not influenced by adjustment for change in systolic blood pressure. The effect of candesartan on LVEF was consistent across predefined subgroups with no significant interaction observed when patients were stratified according to age,

Table 1 Baseline characteristics of the study population

	Candesartan–metoprolol	Candesartan–placebo	Placebo–metoprolol	Placebo–placebo
n	30	32	32	32
Age at recruitment (years)	50.0 ± 8.9	51.7 ± 10.7	50.5 ± 9.1	50.8 ± 9.2
Height (cm)	166.8 ± 6.6	165.5 ± 6.8	167.1 ± 6.1	168.0 ± 5.5
Weight (kg)	70.3 ± 11.3	71.4 ± 14.3	77.7 ± 18.1	72.3 ± 13.7
Systolic blood pressure (mmHg)	124.7 ± 12.8	131.9 ± 14.1*	134.4 ± 13.1**	130.3 ± 12.9
Diastolic blood pressure (mmHg)	78.2 ± 11.5	80.5 ± 8.5	80.5 ± 11.3	80.2 ± 9.9
Heart rate (b.p.m.)	70.8 ± 11.4	71.7 ± 6.7	73.3 ± 10.1	68.3 ± 11.6
Body mass index (kg/m^2)	25.3 ± 3.6	25.9 ± 4.3	27.8 ± 6.3	25.6 ± 4.5
Current smokers	6/30 (20.0%)	7/32 (21.9%)	5/32 (15.6%)	7/32 (21.9%)
Hypertension	1/30 (3.3%)	5/32 (15.6%)	2/32 (6.3%)	0/32 (0%)
Diabetes	0/30 (0%)	1/32 (3.1%)	1/32 (3.1%)	0/32 (0%)
Serum troponin I ≥1.2 ng/L	7/30 (23.3%)	12/32 (37.5%)	9/32 (28.1%)	13/32 (40.6%)
Serum creatinine (mg/dL)	0.75 ± 0.11	0.73 ± 0.10	0.79 ± 0.10	0.74 ± 0.10
Blood haemoglobin (g/dL)	13.2 ± 0.9	13.3 ± 1.0	13.4 ± 0.7	13.2 ± 0.8
Baseline MRI (*n*)	28	32	30	30
Left ventricular ejection fraction (%)	62.2 ± 4.4	62.3 ± 5.3	63.5 ± 5.0	63.6 ± 4.1
Right ventricular ejection fraction (%)	60.6 ± 5.2	60.0 ± 5.2	62.0 ± 4.8	61.2 ± 4.8
Baseline peak systolic global longitudinal strain (*n*)	24	21	23	25
Peak systolic global longitudinal strain	−21.7 ± 1.6	−21.2 ± 1.7	−21.7 ± 2.2	−21.6 ± 1.5
Baseline E/E′ (*n*)	29	30	31	32
E/E′	7.3 ± 2.1	7.5 ± 1.9	6.7 ± 2.1	7.5 ± 1.9
Additional therapy after FEC				
Trastuzumab	7/30 (23.3%)	7/32 (21.9%)	7/32 (21.9%)	7/32 (21.9%)
Radiation	18/30 (60.0%)	19/32 (59.4%)	22/32 (68.8%)	23/32 (71.9%)
Taxanes	25/30 (83.3%)	25/32 (78.1%)	26/32 (81.3%)	24/32 (75%)

Data are expressed as mean ± SD or numbers (per cent).
MRI, magnetic resonance imaging; FEC, 5-fluorouracil, epirubicin, and cyclophosphamide; E/E′, diastolic function.
*$P < 0.05$ for the comparison with candesartan–metoprolol; **$P < 0.01$ for the comparison with candesartan–metoprolol; there were no significant differences between the four study groups, except as noted.

current smoking, history of hypertension, body mass index, radiation, or trastuzumab (*Figure 2A*). No significant effect of candesartan was observed for right ventricular ejection fraction, left ventricular global longitudinal strain, E/E′, cardiac troponin I (*Table 2*), or BNP (Supplementary material online, *Tables S3 and S4*). The effect of candesartan on diastolic function indices is summarized in Supplementary material online, *Table S3*.

In the two groups that were assigned to metoprolol, the mean LVEF reduction was 1.6 (95% CI 0.4, 2.8) percentage points from baseline to the end-of-study, whereas in the two groups assigned to placebo the corresponding decrease was 1.8 (95% CI 0.7, 3.0). This between-group difference was not statistically significant ($P = 0.77$) (*Figure 2B*). There were small but statistically significant increases in E/E′ and BNP levels in the group that received metoprolol compared with the group that did not receive metoprolol. Otherwise, no effect of metoprolol was observed for the secondary outcome measures listed in *Table 2* or the diastolic function indices listed in Supplementary material online, *Table S3*. The effect of metoprolol on heart rate is shown in Supplementary material online, *Figure S3*.

When considering the four randomization groups separately and using the placebo–placebo group as the reference [−2.8 (95% CI −4.3, −1.3)], the reduction in LVEF was significantly less in the candesartan–placebo group than in the placebo–placebo group [−0.9 (95% CI −2.3, 0.4); $P = 0.025$] but not significantly less in the candesartan–metoprolol group than in the placebo–placebo group [−0.6 (95% CI −2.1, 0.8); $P = 0.075$]. No significant difference was observed between the placebo–placebo group and the metoprolol–placebo group [−2.5 (95% CI −3.9, −1.1); $P = 0.71$] (Supplementary material online, *Table S5*).

Compliance, side effects, and serious adverse events

Compliance with study drugs was generally excellent. Two, one, three, and three patients did not adhere to the assigned candesartan, candesartan–placebo, metoprolol, and metoprolol–placebo, respectively, at completion of adjuvant therapy. The mean daily study drug dose at completion of adjuvant therapy was 23 ± 11 mg for candesartan, 26 ± 9 mg for candesartan–placebo, 68 ± 34 mg for metoprolol, and 78 ± 32 mg for metoprolol–placebo. There were no unexpected serious adverse events, the intervention was well tolerated, and no patient in the intention-to-treat analysis

Table 2 Primary and secondary endpoints, estimated values from linear mixed models (intention-to-treat analysis)

	n	Baseline	EOS	Change from baseline to EOS	Between-group difference in change from baseline to EOS	*P*-value
LVEF						
No candesartan	60	63.2 (62.0, 64.4)	60.6 (59.4, 61.8)	−2.6 (−3.8, −1.5)	1.9 (0.2, 3.5)[a]	0.026
Candesartan	60	62.1 (61.0, 63.3)	61.4 (60.2, 62.6)	−0.8 (−1.9, 0.4)		
No metoprolol	62	62.8 (61.6, 64.0)	61.0 (59.8, 62.2)	−1.8 (−3.0, −0.7)	0.2 (−1.4, 1.9)	0.772
Metoprolol	58	62.5 (61.3, 63.7)	61.0 (59.8, 62.2)	−1.6 (−2.8, −0.4)		
RVEF						
No candesartan	60	61.3 (60.0, 62.5)	58.9 (57.6, 60.1)	−2.4 (−3.7, −1.1)	0.8 (−1.0, 2.6)	0.370
Candesartan	60	60.2 (59.0, 61.4)	58.7 (57.4, 59.9)	−1.6 (−2.8, −0.3)		
No metoprolol	62	60.4 (59.2, 61.6)	58.0 (56.8, 59.3)	−2.4 (−3.7, −1.1)	0.8 (−1.0, 2.6)	0.377
Metoprolol	58	61.1 (59.8, 62.3)	59.5 (58.3, 60.8)	−1.6 (−2.9, −0.3)		
LV GLS						
No candesartan	48	−21.6 (−22.1, −21.1)	−21.0 (−21.5, −20.5)	0.6 (0.1, 1.1)	−0.7 (−1.4, 0.1)	0.076
Candesartan	45	−21.3 (−21.8, −20.7)	−21.3 (−21.9, −20.8)	−0.1 (−0.6, 0.5)		
No metoprolol	46	−21.4 (−21.9, −20.8)	−21.0 (−21.6, −20.5)	0.3 (−0.2, 0.8)	−0.1 (−0.8, 0.7)	0.824
Metoprolol	47	−21.5 (−22.0, −21.0)	−21.3 (−21.8, −20.7)	0.2 (−0.3, 0.7)		
E/E′						
No candesartan	63	7.1 (6.6, 7.6)	7.2 (6.7, 7.7)	0.1 (−0.4, 0.5)	0.1 (−0.5, 0.8)	0.688
Candesartan	59	7.4 (6.9, 7.9)	7.6 (7.1, 8.1)	0.2 (−0.2, 0.7)		
No metoprolol	62	7.4 (7.0, 7.9)	7.2 (6.7, 7.7)	−0.3 (−0.7, 0.2)	0.8 (0.2, 1.5)	0.009
Metoprolol	60	7.1 (6.6, 7.5)	7.6 (7.1, 8.1)	0.6 (0.1, 1.0)		
Troponin I[b]						
No candesartan	64	1.1 (0.9, 1.2)	2.7 (2.3, 3.1)	2.5 (2.0, 3.1)	1.1 (0.8, 1.4)	0.666
Candesartan	62	1.0 (0.8, 1.1)	2.5 (2.2, 2.9)	2.6 (2.2, 3.2)		
No metoprolol	64	1.1 (0.9, 1.3)	2.8 (2.4, 3.3)	2.6 (2.1, 3.2)	1.0 (0.7, 1.3)	0.831
Metoprolol	62	0.9 (0.8, 1.1)	2.4 (2.0, 2.8)	2.5 (2.0, 3.1)		

Data are expressed as mean (95% CI).
LVEF, left ventricular ejection fraction; RVEF, right ventricular ejection fraction; LV GLS, left ventricular peak systolic global longitudinal strain; EOS, end-of-study; E/E′, diastolic function.
[a]Rounding effect.
[b]Geometric means.

was withdrawn because of adverse events. Details concerning the serious adverse events are summarized in Supplementary material online, *Table S6*.

Discussion

This randomized, placebo-controlled, double-blind clinical trial demonstrates that in patients with early breast cancer, contemporary anthracycline-containing adjuvant regimens are associated with a numerically modest absolute reduction in left ventricular systolic function and that concomitant administration of the angiotensin receptor blocker candesartan significantly alleviates the decline in LVEF that occurs during adjuvant therapy. Importantly, the effect seemed independent of a direct haemodynamic effect of candesartan as adjustment for change in systolic blood pressure did not impact on the results. No significant beneficial effect of candesartan was observed for the secondary endpoints right ventricular ejection fraction, left ventricular global longitudinal strain, and E/E′, probably reflecting the higher methodological variability of these measurements compared with MRI assessment of LVEF.[23] Candesartan was also ineffective in reducing the increase in circulating cardiac troponin I associated with anthracycline-containing adjuvant therapy, suggesting that angiotensin receptor blockade may not interfere with the direct cardiotoxic effect of anthracyclines, but rather plays a role in the myocardial remodelling process that occurs after cardiac injury.[24]

In contrast to the attenuation of the reduction in left ventricular function observed for candesartan, no short-term beneficial effect was observed for the β-blocker metoprolol. This is in contrast to findings in some prior, small-scale, randomized studies.[14,15,17] Potential reasons for this apparent discrepancy include that patients in prior studies may have received higher doses of anthracyclines and had a higher prevalence of cardiovascular co-morbidities, which could contribute to a favourable effect of β-blockade. Moreover, given that the reduction in LVEF in the placebo−placebo group in our study was less than originally anticipated, the power of the study to detect between-group differences was reduced. Accordingly, the apparent lack of effect of metoprolol on LVEF may also be due to inadequate statistical power and does not rule out a beneficial effect

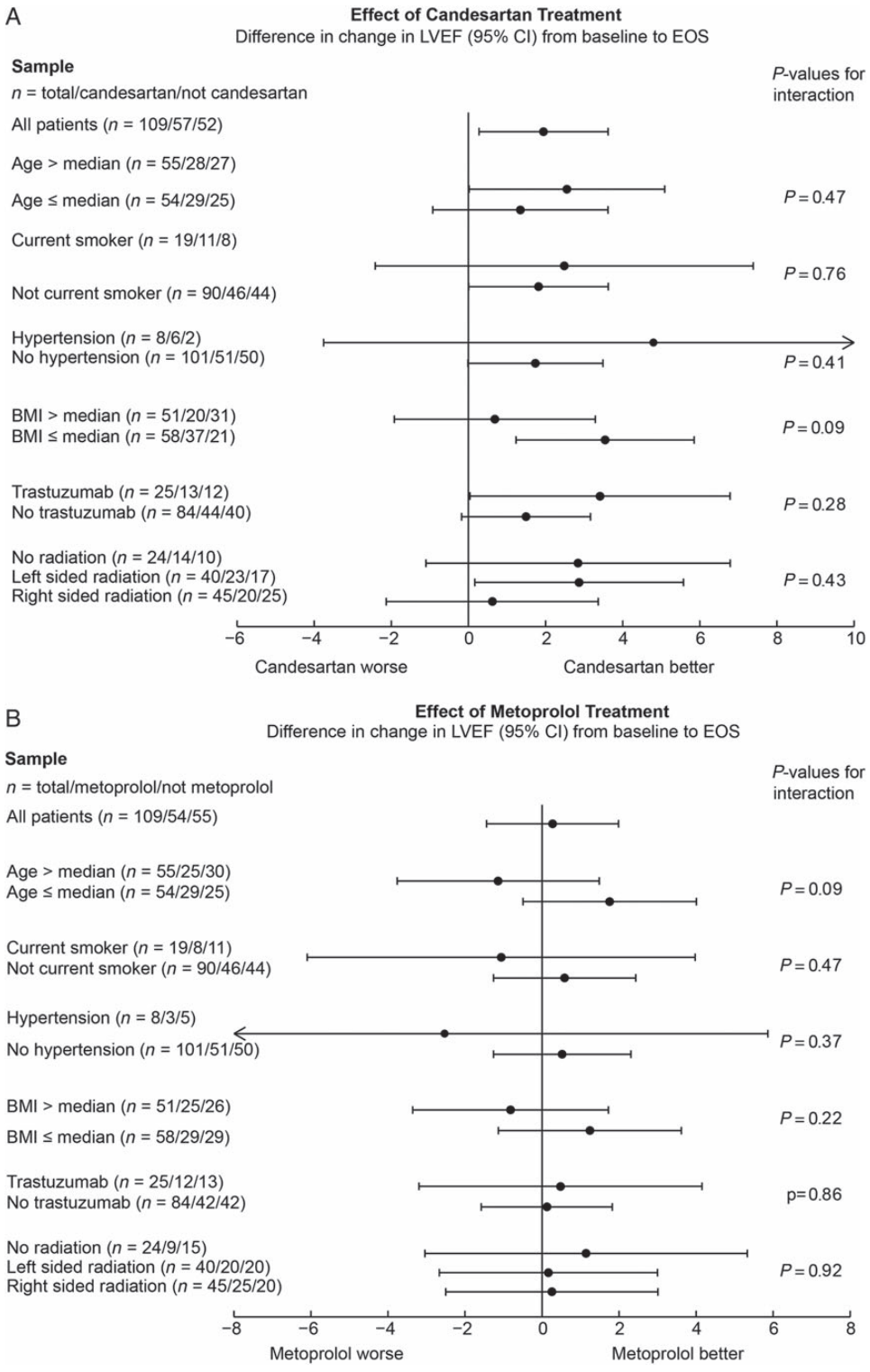

Figure 2 Effect of candesartan and metoprolol on left ventricular ejection fraction during adjuvant therapy for early breast cancer. Shown are the changes in left ventricular ejection fraction expressed in percentage points with 95% confidence intervals. Concomitant therapy with candesartan alleviated the decline in left ventricular ejection fraction observed in the placebo group. This effect was consistent across subgroups with no formal interaction observed when patients were stratified according to age, current smoking, history of hypertension, body mass index, trastuzumab, or radiation (*A*). No effect of metoprolol on the mean left ventricular ejection fraction was observed (*B*). Median age at baseline was 49 years, and median body mass index at baseline was 25.6 kg/m^2. EOS, end-of-study; LVEF, left ventricular ejection fraction by magnetic resonance imaging; BMI, body mass index.

of β-blockade. Finally, we cannot rule out the possibility that an alternative β-blocker or a higher dose would have proved effective.

Although some studies have indicated that echocardiographic indices of diastolic function may detect subclinical changes in cardiac function during cancer treatment, their predictive value remains unproven.[23,25] Moreover, in the oncological setting, changes in diastolic indices such as the E/E′ ratio could be the result of changes in loading conditions secondary to the nausea and vomiting commonly associated with chemotherapy.[25] We observed a small increase in E/E′, an index closely associated with left ventricular filling, in the metoprolol but not in the no-metoprolol group. This increase is likely associated with a direct haemodynamic effect of β-blockade.[26] Similarly, BNP concentrations increased in the metoprolol group but remained unchanged in the candesartan group during adjuvant chemotherapy. It is well documented that β-blockade, via its effects on heart rate and stroke volume, causes increased release of natriuretic peptides.[27] Accordingly, in the absence of development of symptomatic ventricular dysfunction, it is not surprising that metoprolol is associated with an increase in BNP levels in the current study. The lack of effect of candesartan on BNP can probably be accounted for by its relatively high intra- and inter-individual variability[28] and is in accordance with other recent studies examining the effect of anthracycline therapy on BNP.[29]

The current results may have potential important implications. A reduction in LVEF is commonly considered a late-occurring phenomenon in the cardiotoxic process, manifesting itself first after myocardial reserves are exhausted.[1] This study, using the reference method for assessment of left ventricular function, demonstrates that low-to-moderate doses of anthracyclines with or without trastuzumab or radiation are associated with a numerically modest, but significant reduction in LVEF that was somewhat less than that we *a priori* had defined as a clinically important difference. This observation is in accordance with another recent, smaller ($n = 58$ with cardiac MRI imaging) randomized, controlled, but non-blinded trial of malignant haemopathies receiving anthracycline-based chemotherapy that found an absolute reduction of LVEF of 3.0 percentage points in the placebo group.[14] Moreover, our findings are in accordance with those of an observational study using cardiac MRI in a more heterogeneous population of cancer patients ($n = 53$) treated with low-to-moderate dose anthracycline-based chemotherapy.[2] Although the latter study included patients with prior coronary artery disease and a high proportion of patients had hypertension (40%) and other cardiovascular risk factors, the absolute reduction in LVEF was only moderately higher than in the current, all-female previously healthy study population. Taken together, these studies consistently show that contemporary doses of anthracycline-containing chemotherapy regimens are associated with a modest, but highly statistically significant reduction of LVEF, but that development of severe ventricular dysfunction is a rare-occurring event in the short term.

A crucial question, however, is whether these numerically modest early changes in LVEF and the prevention of early decline in ventricular function may have any consequences for the long-term risk of developing more severe asymptomatic or symptomatic ventricular dysfunction. As imaging methods used in the past may have lacked the precision to identify minor LVEF changes, the long-term implications of reduction in LVEF following the exposure to cardiotoxic agents are not yet fully known, but it is well documented that the process of left ventricular dysfunction after other types of myocardial injury is progressive and early intervention is crucial to prevent deterioration in the long term. The notion of the importance of early intervention is also supported by observational data, suggesting that the duration from completion of high-dose anthracycline therapy to initiation of angiotensin-converting enzyme inhibition is a key determinant of the magnitude of the beneficial effect.[30] This was recently highlighted by Cardinale *et al.*, who in a prospective study of 2625 anthracycline-receiving patients reported an association between end-of-chemotherapy LVEF and cardiotoxicity development.[6] In our study, concomitant treatment with candesartan prevented the early LVEF decline associated with adjuvant therapy for breast cancer. Accordingly, it seems likely that this attenuation of the early decline in ventricular function may have beneficial long-term consequences concerning the risk of developing asymptomatic or symptomatic ventricular dysfunction.

Strengths of the current study include the 2 × 2 factorial design, permitting a head-to-head comparison of two different drugs, the use of serial cardiac MRI investigations in a homogeneous cohort of patients with breast cancer treated with contemporary adjuvant therapy, including low-to-moderate doses of epirubicin. Accordingly, our results are generalizable to a large number of women with early breast cancer. Using a method with low variability, the current trial had a high likelihood to detect even modest differences between groups. Limitations of the current report include the lack of follow-up information beyond the adjuvant therapy period, but long-term follow-up of the participants with repeat cardiac MRI investigations is planned. We excluded some patients at high cardiovascular risk, but many of these, including those with prior cardiovascular disease, had indications for treatment with β-blockers or inhibitors of the renin–angiotensin system. The dose of metoprolol attained was moderately high, but resulted in a significant reduction in heart rate compared with the placebo group, suggesting good compliance and adequate β-blockade. Although predefined subgroup analyses showed a consistent effect across subgroups, including those who received higher dose anthracyclines and trastuzumab, the statistical power to conduct subgroup analyses in this study is limited, and this observation must be verified in adequately powered trials with long-term follow-up.

In conclusion, using cardiac MRI we found that adjuvant breast cancer treatment is associated with a decline in LVEF that is alleviated by concomitant neurohormonal blockade with candesartan. Long-term follow-up of these patients will document whether the beneficial effect of candesartan is sustained and will translate into reduced incidence of left ventricular dysfunction.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Authors' contributions

M.W.F. performed statistical analysis. T.O., J.G., K.S., P.H., T.H.S., J.S.-M., and F.K.-B. handled funding and supervision. G.G., S.L.H., B.G., Å.B., and H.R. acquired the data. T.O., A.H.R., and J.G. conceived and designed the research. T.O., G.G., and S.L.H. drafted

the manuscript. A.H.R., P.H., J.S.-M., M.W.F., B.G., F.K.-B., Å.B., T.H.S., T.-A.H., H.R., K.S., and J.G. made critical revision of the manuscript for key intellectual content. T.-A.H. was responsible for the biochemical analysis.

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Conflict of interest: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. T.O. has served on advisory boards for Abbott Diagnostics and Novartis, has received research support from AstraZeneca and Abbott Diagnostics via Akershus University Hospital, and speakers' honoraria from Roche Diagnostics. No other disclosure were reported.

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Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 x 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol

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The work was performed at Akershus University Hospital.

Contents

Details on patient inclusion

Between September 2011 and September 2014, 130 patients with early breast cancer having surgery at Department of Surgery at Akershus University Hospital and scheduled for adjuvant therapy with the anthracycline epirubicin were enrolled in the trial and assigned to one of the four treatment groups. Four patients were characterized as randomization failures (two did not receive planned adjuvant treatment, one was discovered to have been previously treated with radiation therapy and one was discovered to likely have had cardiovascular complication in the pre-randomization phase), leaving 126 patients in the study population. Six patients did not undergo cardiac MRI, leaving 120 patients to be included in the intention-to-treat analysis of the primary endpoint.

Details on randomization

A permuted block randomization procedure with undisclosed and variable blocking factor 4:8 were used to generate the randomized list which was created by a statistician from Oslo Centre for Biostatistics and Epidemiology at Oslo University Hospital Patients were stratified according to trastuzumab therapy. Sealed, opaque envelopes with the treatment codes were stored in a locked cabinet in the offices of the Department of Clinical Research, Division of Medicine, Akershus University Hospital. Tablets with active substance and respective placebos had identical appearances. Both study personnel and participants were unaware of treatment assignments, i.e. the study was double-blind. In addition, cardiac MRI and echocardiographic image analyses were performed with the investigators blinded to patient identity and image sequence. Statistical analyses were performed with the statistician blinded to treatment intervention group.

Details on dose titration

Dose titration was performed as follows: Candesartan cilexetil/placebo starting dose 8 mg q.d; Metoprolol succinate/placebo starting dose 25 mg q.d. Provided no symptoms of hypotension/and or bradycardia after on average 3 days, study drug doses were uptitrated to candesartan/placebo 16 mg q.d. and metoprolol/placebo 50 mg q.d. Provided no symptoms of hypotension/and or bradycardia after 3 additional days, study drug doses were uptitrated to candesartan/placebo 32 mg q.d. and metoprolol/placebo 100 mg q.d. If signs or symptoms of

hypotension occurred, dosage of both medications was reduced. If reduction of only one medication was indicated, the metoprolol/placebo dose was reduced first. If signs or symptoms of bradycardia occurred, only the metoprolol/placebo dose was reduced.

Details on MRI analysis

Cardiac MRI analysis was performed on dedicated, commercially available software (cvi42, Circle Cardiovascular Inc. Calgary, Canada). Epicardial and endocardial contours were traced in end-diastole and end-systole according to Society for Cardiovascular Magnetic Resonance guidelines, permitting calculation of ventricular volumes, ejection fraction and left ventricular mass. Trabeculations and papillary muscles were included in the ventricular volumes and excluded from the calculations of left ventricular mass.

Intra-observer variability for LVEF was assessed in a randomly selected sample of 15 patients. For validation, the same sample was also assessed for inter-observer variability with expert readers at Medical University Berlin, Charité Campus Buch. The intra-observer LVEF intra-class correlation coefficient was 0.96 (95 % confidence interval 0.88, 0.99). The inter-observer LVEF intra-class correlation coefficient was 0.91 (95 % confidence interval 0.73, 0.94). The mean intra-observer difference for LVEF was -0.7±1.6 percentage points. The mean inter-observer difference for LVEF was -0.8±2.4 percentage points.

Details on echocardiographic analysis

Two- and three-dimensional images and loops were acquired with a 2.5-MHz transducer and a 4-volt matrix-array transducer, respectively. In accordance with the European Association of Cardiovascular Imaging[1], standard parasternal long axis and apical view recordings were done in the end-expiratory phase with the subjects in the supine left lateral position. The average of three cycles was used for standard measurements of cardiac function and dimensions. Biplanar apical 2- and 4-chamber views were used to measure left atrial volume by the area length method. All measurements were performed in end-systole. In addition to E/E` left ventricular diastolic function was assessed by mitral inflow velocities including the ratio between peak early (E) and atrial (A) velocities and the deceleration time by pulsed Doppler.[2] Specific 3-dimensional loops were recorded from the apical view by storing four heart cycles and offline analyses of LV volumes (TomTec Imaging Systems, Germany).

Supplemental tables

Table S1: Eligibility criteria

Inclusion criteria

Women aged 18-70 years

Eastern Cooperative Oncology Group (ECOG) performance status 0–1

Serum creatinine < 1.6 mg/dL or estimated glomerular filtration rate (eGFR) ≥ 60 ml/min/1.73 m^2

Systolic blood pressure ≥ 110 mmHg and < 170 mmHg

Left ventricular ejection fraction ≥ 50%

Exclusion criteria

Hypotension, defined as systolic blood pressure < 110 mmHg

Prior anthracycline chemotherapy regimen

Prior malignancy requiring chemotherapy or radiotherapy

Symptomatic heart failure

Systolic dysfunction (left ventricular ejection fraction < 50%)

Clinically significant coronary artery disease, valvular heart disease, significant arrhythmias, or conduction delays

Bradycardia, defined as heart rate < 50 beats per minute

Uncontrolled arterial hypertension defined as systolic blood pressure > 170 mmHg

Treatment with angiotensin-converting enzyme inhibitor, angiotensin receptor blocker or beta-blocker within the last 4 weeks prior to study start

Intolerance to angiotensin-converting enzyme inhibitor, angiotensin receptor blocker or beta-blocker

Uncontrolled concomitant serious illness, as determined by the investigator

Pregnancy or breastfeeding

Active abuse of drugs or alcohol

Suspected poor compliance

Inability to tolerate the MRI protocol

Table S2: Tumour characteristics

	Candesartan-metoprolol	Candesartan-placebo	Placebo-metoprolol	Placebo-placebo
N	30	32	32	32
Mastectomy	8 (26.7%)	8 (25%)	15 (46.9%)	16 (50%)
Right Side	17 (57.6%)	16 (50%)	20 (62.5%)	17 (53.1%)
Left side	12 (40%)	16 (50%)	10 (31.3%)	15 (46.9%)
Bilateral	1 (3.3%)	0 (0%)	2 (6.3%)	0 (0%)
Tumour size:				
Tumour not found	0 (0%)	2 (6.3%)	0 (0%)	0 (0%)
T1	15 (50%)	17 (53.1%)	17 (53.1%)	12 (37.5%)
T2	15 (50%)	12 (37.5%)	15 (46.9%)	19 (59.4%)
T3	0 (0%)	1 (3.1%)	0 (0%)	1 (3.1%)
Tumour grade				
G0	0 (0%)	1 (3.1%)	0 (0%)	0 (0%)
G1	2 (6.7%)	2 (6.3%)	0 (0%)	2 (6.3%)
G2	12 (40%)	11 (34.4%)	14 (43.8%)	9 (28.1%)
G3	16 (53.3%)	18 (56.3%)	18 (56.3%)	21 (65.6%)
Lymph node				
N0	16 (53.3%)	20 (62.5%)	18 (56.3%)	17 (53.1%)
N1	12 (40%)	7 (21.9%)	7 (21.9%)	11 (34.4%)
N2	2 (6.7%)	5 (15.6%)	6 (18.8%)	2 (6.3%)
N3	0 (0%)	0 (0%)	1 (3.1%)	2 (6.3%)
Immunohistology				
HER 2 positive*	7 (23.3%)	7 (21.9%)	7 (21.9%)	8 (25%)
Oestrogen Receptor negative (< 1%)	7 (23.3%)	8 (25%)	6 (18.8%)	10 (31.3%)
Oestrogen Receptor positive >1% and <50%	1 (3.3%)	0 (0%)	4 (12.5%)	3 (9.4%)
Oestrogen Receptor positive ≥50%	22 (73.3%)	24 (75%)	22 (68.8%)	19 (59.4%)
Progesterone Receptor positive (>10%)	18 (60%)	20 (62.5%)	18 (56.3%)	19 (59.4%)
Ki 67 ≥ 30 %	16 (53.3%)	22 (68.8%)	18 (56.3%)	17 (53.1%)
Ki 67 < 30%	5 (16.7%)	7 (21.9%)	10 (31.3%)	8 (25%)
Not measured	9 (30%)	3 (9.4%)	4 (12.5%)	7 (21.9%)
Treatment				
FEC 240 mg/m² †	18 (60%)	19 (59.4%)	17 (53.1%)	17 (53.1%)
FEC 360 mg/m² †	5 (16.7%)	6 (18.8%)	6 (18.8%)	6 (18.8%)
FEC 400 mg/m² †	7 (23.3%)	7 (21.9%)	7 (21.9%)	7 (21.9%)
Incomplete FEC treatment	0 (0%)	0 (0%)	2 (6.3%)	2 (6.3%)
Trastuzumab	7 (23.3%)	7 (21.9%)	6 (18.8%)	7 (21.9%)
Incomplete Trastuzumab	0 (0%)	0 (0%)	1 (3.1%)	0 (0%)
Taxol	22 (73.3%)	22 (68.8%)	22 (68.8%)	17 (53.1%)
Taxotere	1 (3.3%)	1 (3.1%)	2 (6.3%)	4 (12.5%)
Incomplete Taxane treatment	2 (6.7%)	2 (6.3%)	2 (6.3%)	3 (9.4%)
Radiation	18 (60%)	19 (59.4%)	22 (68.8%)	23 (71.9%)
Left side	5/18 (27.8%)	11/19 (57.9%)	6/22 (27.3%)	8/23 (34.8%)
Right side	13/18 (72.2%)	8/19 (42.1%)	16/22 (72.7%)	15/23 (65.2%)

* HER 2 positive denotes IHC 3+ or IHC 2+ and amplified (ISH)

† Accumulated epirubicin dose

There were no significant differences between the four study groups

Table S3: Indices of left ventricular diastolic function from linear mixed models (ITT analysis)

	n	Baseline		EOS		Change from baseline to EOS		Between-group difference in change from baseline to EOS		p-value
E/E'										
No candesartan	63	7.1	(6.6, 7.6)	7.2	(6.7, 7.7)	0.1	(-0.4, 0.5)	0.1	(-0.5, 0.8)	0.688
Candesartan	59	7.4	(6.9, 7.9)	7.6	(7.1, 8.1)	0.2	(-0.2, 0.7)			
No metoprolol	62	7.4	(7.0, 7.9)	7.2	(6.7, 7.7)	-0.3	(-0.7, 0.2)	0.8	(0.2, 1.5)	0.009
Metoprolol	60	7.1	(6.6, 7.5)	7.6	(7.1, 8.1)	0.6	(0.1, 1.0)			
E'										
No candesartan	64	0.12	(0.11, 0.12)	0.11	(0.11, 0.12)	-0.00	(-0.01, 0.00)	0.00	(-0.01, 0.01)	0.732
Candesartan	61	0.11	(0.11, 0.12)	0.11	(0.10, 0.12)	-0.00	(-0.01, 0.00)			
No metoprolol	63	0.12	(0.11, 0.12)	0.11	(0,11, 0.12)	-0.00	(-0.01, 0.00)	-0.00	(-0.01, 0.00)	0.306
Metoprolol	62	0.12	(0.11, 0.12)	0.11	(0.10, 0.12)	-0.01	(-0.01, -0.00)			
E/A										
No candesartan	63	1.33	(1.25, 1.41)	1.35	(1.27, 1.44)	0.02	(-0.06, 0.10)	0.04	(-0.07, 0.15)	0.518
Candesartan	59	1.27	(1.19, 1.35)	1.33	(1.25, 1.41)	0.06	(-0.02, 0.14)			
No metoprolol	62	1.32	(1.24, 1.40)	1.32	(1.23, 1.40)	-0.00	(-0.08, 0.08)	0.09	(-0.03, 0.20)	0.134
Metoprolol	60	1.28	(1.20, 1.36)	1.37	(1.28, 1.45)	0.08	(0.01-0.16)			
Deceleration time ms										
No candesartan	63	198	(187, 208)	198	(186, 210)	1	(-15, 16)	7	(-14, 28)	0.529
Candesartan	59	198	(187, 209)	205	(194, 217)	7	(-7, 22)			
No metoprolol	62	198	(187, 209)	203	(191, 214)	5	(-10, 20)	-2	(-23, 19)	0.883
Metoprolol	60	198	(187, 209)	201	(189, 213)	3	(-12, 18)			
LAESV mL/m^2										
No candesartan	55	24.0	(22.4, 25.7)	23.3	(21.6, 25.1)	-0.7	(-2.5, 1.1)	2.8	(0.3, 5.3)	0.031
Candesartan	49	23.3	(21.7, 25.0)	25.4	(23.8, 27.1)	2.1	(0.3, 3.9)			
No metoprolol	53	24.0	(22.3, 25.6)	23.9	(22.2, 25.5)	-0.1	(-1.9, 1.6)	1.6	(-0.9, 4.2)	0.205
Metoprolol	51	23.4	(21.7, 25.1)	25.0	(23.3, 26.7)	1.5	(-0.3, 3.3)			

Data are expressed as mean (95% CI); ITT, intention to treat; EOS, end-of-study; E/E`, ratio between peak early (E) transmitral velocity by pulsed Doppler and peak early tissue Doppler (E`); E/A, ratio between peak early (E) and atrial (A) velocities by pulsed Doppler; Deceleration time by pulsed Doppler; LAESV, left atrial end-systolic volume;

Table S4: BNP, estimated values from linear mixed models (ITT analysis)

	n	Baseline		EOS		Change from baseline to EOS		Between-group difference in change from baseline to EOS		p-value
BNP pg/mL										
No candesartan	63	15.6	(11.4, 19.9)	19.5	(15.2, 23.9)	3.9	(-1.0, 8.9)	1.9	(-5.0, 8.8)	0.594
Candesartan	62	14.0	(9.7, 18.3)	19.9	(15.6, 24.2)	5.8	(1.0, 10.7)			
No metoprolol	64	12.3	(8.1, 16.5)	13.6	(9.3, 17.9)	1.3	(-3.6, 6.1)	7.3	(0.4, 14.3)	0.038
Metoprolol	61	17.4	(13.1, 21.7)	26.0	(21.6, 30.4)	8.6	(3.7, 13.5)			

Data are expressed as mean (95% CI); BNP, B-type natriuretic peptide; ITT, intention to treat; EOS, end-of-study

Table S5: Primary outcome within each of the four treatment group combinations; estimated values from linear mixed models with interaction term between candesartan and metoprolol

	n	Baseline	EOS	Change	P-value against placebo-placebo
Placebo-placebo	30	63.1 (61.5, 64.8)	60.3 (58.6, 62.1)	-2.8 (-4.3, -1.3)	Reference
Candesartan-placebo	32	62.5 (60.9, 64.1)	61.6 (60.0, 63.1)	-0.9 (-2.3, 0.4)	0.025
Metoprolol-placebo	30	63.3 (61.7, 64.9)	60.8 (59.2, 62.5)	-2.5 (-3.9, -1.1)	0.71
Candesartan-metoprolol	28	61.7 (60.0, 63.4)	61.1 (59.4, 62.8)	-0.6 (-2.1, 0.8)	0.075

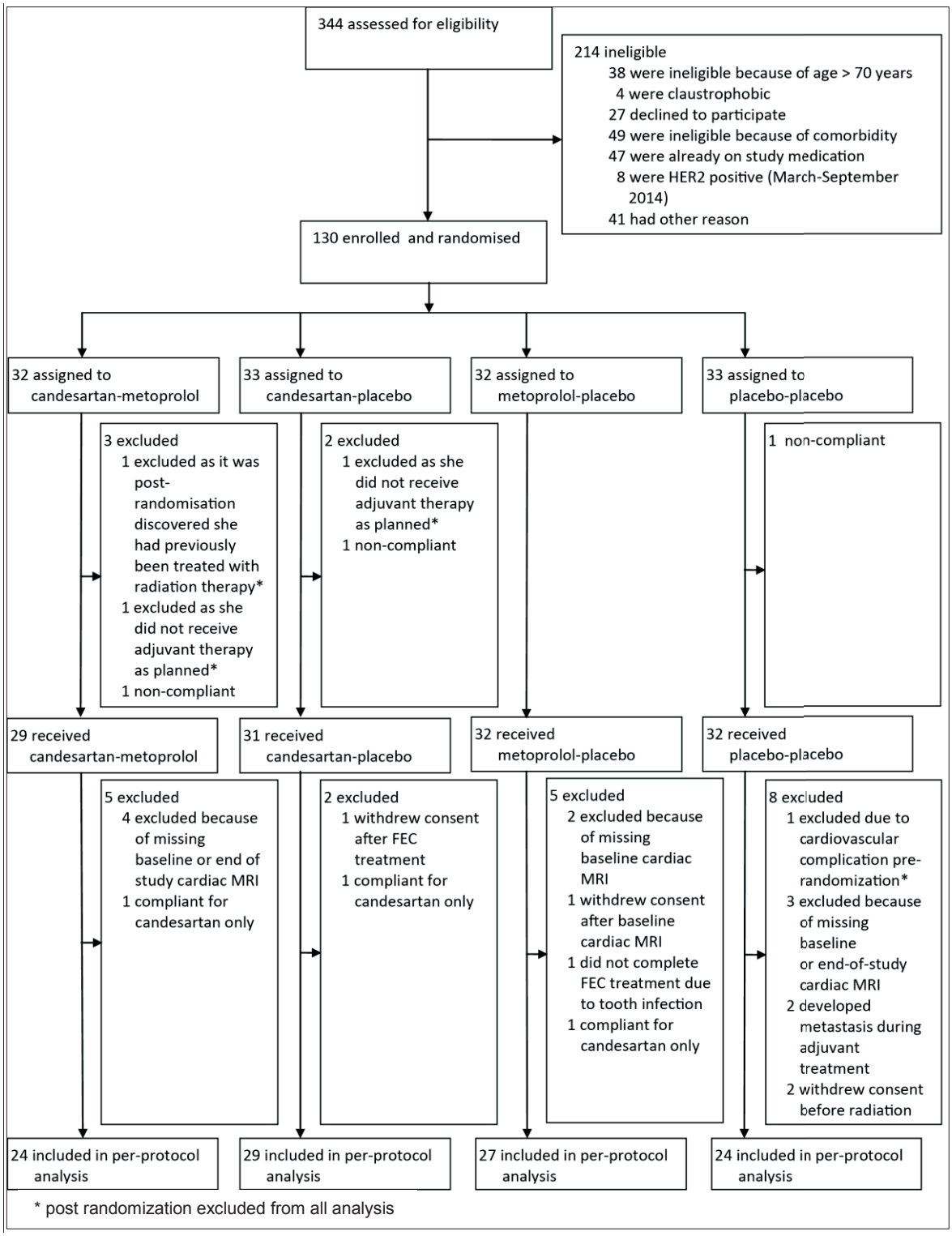
Left ventricular ejection fraction (95% CI); EOS, end-of-study.

Table S6: Incidence of serious adverse events by treatment arm

Treatment Arm	Number of Serious Adverse Events	Type of Serious Adverse Events
Candesartan-metoprolol	2	Thrombus in right atrium, vasovagal syncope
Candesartan-placebo	2	Loss of muscle tonus/epilepsy, deep venous thrombosis
Metoprolol-placebo	4	Rash (1 week before end of study), nasopharyngitis, thrombophlebitis, pneumonia
Placebo-placebo	1	Depression

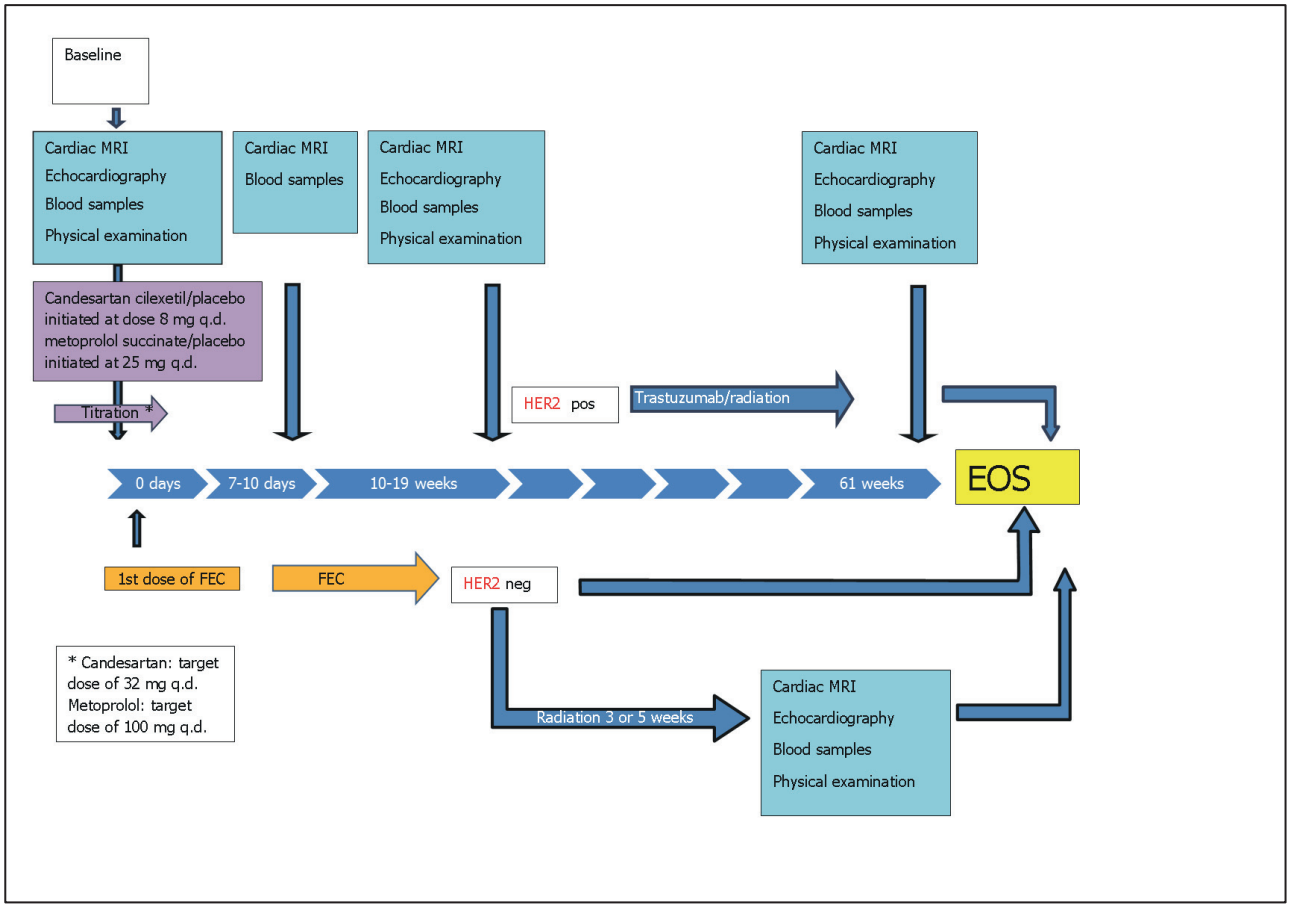
Supplemental figures

Figure S1: Screening criteria and randomization for per-protocol patients



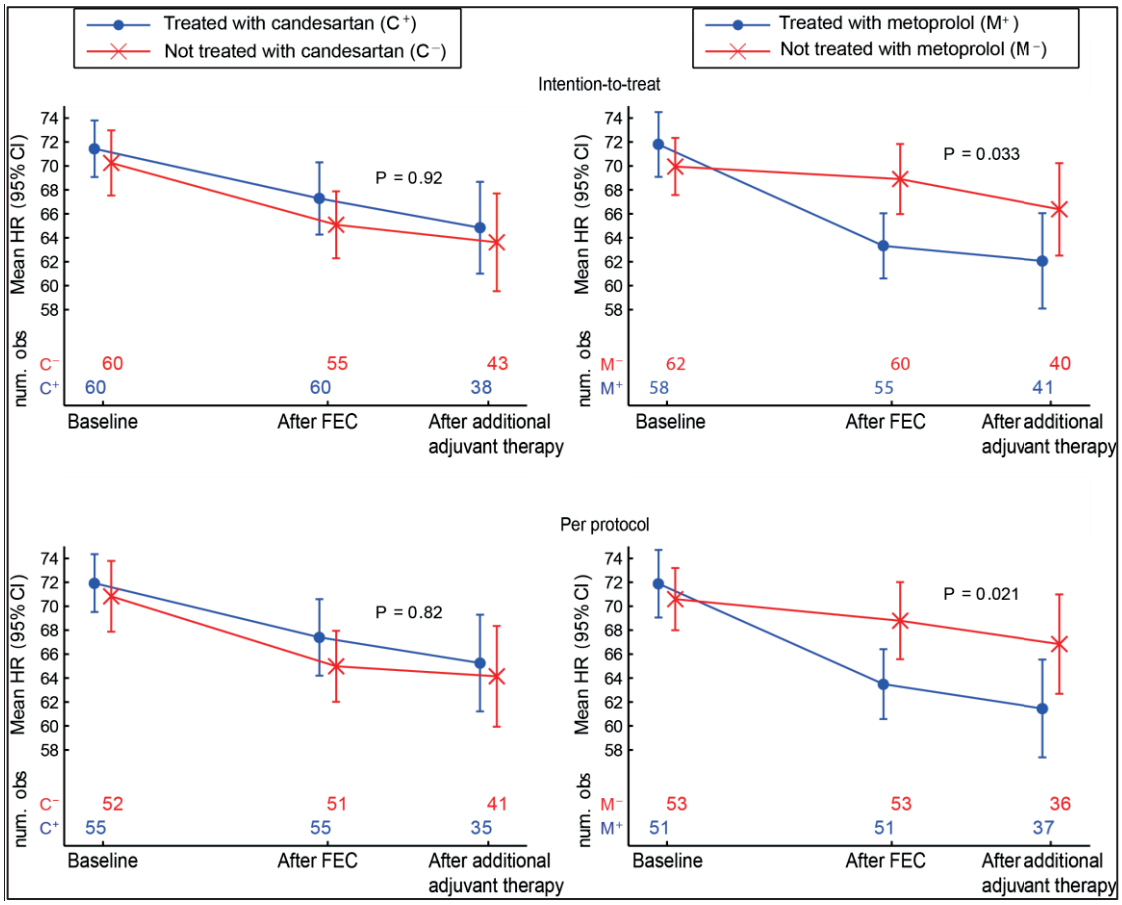
The per-protocol-analysis excluded patients who did not have baseline and end-of-study MRI measurements, discontinued their study medication, withdrew consent or did not complete adjuvant therapy.
HER, Human Epidermal Growth Factor Receptor; FEC, 5-Fluorouracil, Epirubicin and Cyclophosphamide; MRI; Magnetic Resonance Imaging.

Figure S2: PRADA study flow sheet



Flow chart for the PRADA study showing data obtained at different time points during the study and that the time to end-of-study differs according to anti-cancer treatment. MRI, Magnetic Resonance Imaging; EOS End-of-Study; FEC, 5-Fluorouracil, Epirubicin and Cyclophosphamide; HER, Human Epidermal Growth Receptor; neg, negative; pos, positive.

Figure S3: Effect of metoprolol and candesartan on heart rate



The figure shows the longitudinal heart rate response to candesartan and metoprolol vs. placebo. HR, Heart Rate; CI, Confidence Interval; num. obs, number of observations; FEC, 5-Fluorouracil, Epirubicin and Cyclophosphamide.

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III

IV

Neurohormonal Blockade and Circulating Cardiovascular Biomarkers During Anthracycline Therapy in Breast Cancer Patients: Results from The PRADA Study

Short Title

Gulati: Cardiac biomarkers during cancer therapy

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ABSTRACT

Background: Anthracyclines are associated with cardiotoxic effects. Cardiovascular biomarkers may reflect myocardial injury, dysfunction, inflammation and fibrosis and may precede and predict the development of left ventricular impairment. The aim of this study was to assess: (1) longitudinal change in circulating cardiovascular biomarkers**,** (2) the effect of metoprolol succinate and candesartan cilexetil on the biomarker response and (3) the associations between on-treatment changes in biomarker concentrations and subsequent left ventricular dysfunction in early breast cancer patients receiving anthracyclines.

Methods and results: This report encompasses 121 women included in the 2x2 factorial, placebo-controlled, double-blind PRevention of cArdiac Dysfunction during Adjuvant breast cancer therapy (PRADA) trial with metoprolol and candesartan given concomitantly with anticancer therapy containing the anthracycline epirubicin (total cumulative dose 240-400 mg/m^2). Cardiovascular magnetic resonance, echocardiography images and circulating levels of biomarkers were obtained before and after anthracycline treatment. Cardiac troponins (cTn) I and T, B-type natriuretic peptide, N-terminal pro-B-type natriuretic peptide, C-reactive protein, and galectin-3 increased during anthracycline therapy (all $p<0.05$). The troponin response was attenuated by metoprolol ($p<0.05$) but not candesartan. There was no association between change in biomarker concentrations and change in cardiac function during anthracycline therapy.

Conclusions: Treatment with contemporary anthracycline doses for early breast cancer is associated with increase in circulating cardiovascular biomarkers. This increase is however not associated with early decline in ventricular function. Beta-blockade may attenuate early myocardial injury, but whether this attenuation translates into reduced risk of developing ventricular dysfunction in the long term remains unclear.

Clinical Trial Registration: ClinicalTrial.gov number NCT01434134.

Key words: cardio-oncology, cardiovascular biomarkers, candesartan, metoprolol

Introduction

Anthracyclines are frequently used in the treatment of several common malignancies, including breast cancer. However, anthracyclines have well-known cardiotoxic effects leading to myofibrillar degradation and cardiomyocyte apoptosis and necrosis[1]. Different strategies to reduce the cardiotoxicity, including lower peak and cumulative drug doses, have been implemented, but contemporary doses still increase the risk of developing left ventricular systolic dysfunction[2].

Circulating cardiovascular biomarkers may reflect pathophysiological processes that play a crucial role for cardiotoxicity, including cardiomyocyte injury, function, inflammation and fibrosis. High-dose anthracycline therapy has been associated with increased concentrations of cardiac troponins[3] and B-type natriuretic peptides[4], but in more recent studies with contemporary anthracycline doses, results have been inconsistent[5]. Prior studies of patients receiving high dose anthracyclines have also suggested that the initial response in these biomarkers may predict subsequent decrease in left ventricular function[6]. However, in breast cancer patients receiving contemporary doses of anthracyclines sparse data are available concerning the prognostic value of early changes in cardiovascular biomarkers. Other cardiac biomarkers such as C-reactive protein (CRP) and galectin-3 are thought to reflect systemic inflammation and fibrosis, but the prognostic value in breast cancer patients has been less investigated.

Decline in left ventricular ejection fraction is the established imaging marker for cardiotoxicity[7]. Cardiovascular magnetic resonance (CMR) is also an excellent modality to detect focal fibrosis in ischemic and non-ischemic cardiomyopathy and edema during and after acute myocardial injury[8]. Although a recent meta-analysis indicates that intervention with beta blockers and angiotensin antagonists prevents or delays the development of left ventricular (LV) dysfunction in early-onset anthracycline-induced cardiotoxicity[9], there are limited data on how

this intervention affects circulating levels of cardiovascular biomarkers. The aim of this substudy of the PRADA (PRevention of cArdiac Dysfunction during Adjuvant breast cancer therapy) trial was therefore to (i) longitudinally examine the circulating profile of the biomarkers cardiac troponin I and T (cTnI and cTnT), B-type natriuretic peptide (BNP) and the amino-terminal fragment of the BNP prohormone (NT-proBNP), CRP and galectin-3, (ii) assess the effect of the angiotensin receptor blocker candesartan and the beta blocker metoprolol on the biomarker response and (iii) evaluate the association between changes in these biomarker and subsequent reduction in left ventricular systolic function during anthracycline treatment in early breast cancer patients.

Methods

Study design

PRADA was an investigator-initiated, externally-monitored 2x2 factorial, randomized, placebo-controlled double blind trial conducted at Akershus University Hospital, Lørenskog, Norway[10]. Patients were randomized to one of four combinations of intervention as following: 29 in the metoprolol succinate and placebo arm, 32 in the candesartan cilexetil and placebo arm, 30 in the metoprolol and candesartan arm and 30 in the double placebo arm. The target dose for metoprolol was 100 mg q.d and candesartan 32 mg q.d. The study complied with the declaration of Helsinki. The study protocol was approved by the Regional Ethics Committee of South-Eastern Norway (2010/2890) and registered at ClinicalTrials.gov (NCT01434134). All participants provided written informed consent. The rationale and design of the study has been published previously[10, 11].

Study participants

In total, 130 women with early breast cancer scheduled for adjuvant therapy with the anthracycline epirubicin in combination with 5-fluorouracil and cyclophosphamide (FEC) were included at Akershus University Hospital, Norway from September 2011 to September 2014. Main exclusion criteria were pre-existing cardiovascular disease, previous treatment with chemotherapy or radiation to the chest and indication or contraindications for the study drugs. Post randomization four patients were excluded; two did not receive planned adjuvant treatment, one had previously been treated with radiation to the chest and one likely had experienced a cardiovascular complication in the pre-randomization phase. In this report additionally five patients were excluded, four did not complete their chemotherapy regimen as planned, and one did not have biomarker measurements at completion of anthracycline treatment. Hence, 121 patients constitute the study population of this report. The exact timing of blood sampling and cardiac imaging is shown in Figure 1. All data were obtained pre- and post anthracycline treatment, and before initiation of additional therapy with trastuzumab or radiotherapy.

Cardiovascular magnetic resonance and echocardiography

The CMR and echocardiography methodologies have been described in details previously[10]. In brief, CMR examinations were performed on a 1.5-T scanner (Achieva; Philips Medical Systems, Best, The Netherlands). Steady-State-Free-Precession sequences in contiguous, eight mm thick short-axis slices covering the entire ventricles were used to quantify left ventricular ejection fraction (LVEF). Cardiac edema was assessed by breath-hold, black-blood triple inversion recovery T2 imaging in three 15 mm short axis slices of the LV. TR/TE/flip angle were 2 heartbeats/65 ms/90°, acquired and reconstructed voxel size were 1.5/1.9/15 mm^3 and 0.7x0.7x15mm^3, respectively. Late gadolinium enhancement (LGE) images were acquired 10

min after intravenous injection of 0.2 mmol/kg Gadolinium-DOTA (Dotarem®, Guerbet, France). Typically, 2-dimensional inversion recovery turbo field echo sequence in short axis covering the ventricles, and phase-sensitive 3-dimensional inversion recovery turbo field echo sequences in four chamber and left two chamber axis were used. For the 2D scans, TR/TE/flip angle were 5.8 ms/2.9 ms/25°, acquired voxel size was 1.5x1.6x8mm^3 and reconstructed voxel size 0.8x0.8x8mm^3. For the 3D scans, TR/TE/flip angle were 4.8ms/2.3ms/15° and acquired and reconstructed voxel sizes were 2.0x2.0x10mm^3 and 1.3x1.3x5.5mm^3, respectively. Analyses were performed by a board-certified radiologist (SLH), who was blinded to treatment assignment and study order.

Transthoracic echocardiography was performed using a Vivid E9 (GE Vingmed, Horten, Norway). Images were digitally stored for offline analysis on custom software (EchoPAC, GE Vingmed, Horten, Norway). Left ventricular two-dimensional peak systolic global longitudinal strain was analyzed by a semi-automated speckle-tracking imaging technique from the three standard apical views. Left ventricular diastolic function was assessed by the ratio between peak early (E) transmitral velocity by pulsed Doppler and peak early tissue Doppler (E`) by averaging septal and lateral E` at the base of septal and mitral leaflet, respectively. Analyses were performed by a board-certified physician (GG), who was blinded to treatment assignment and study order.

Blood sampling and biochemical analysis

Non-fasting samples of venous blood were drawn, put on ice, processed within 60 minutes, and stored at −80° C pending analysis. Before analysis, thawed specimens were mixed thoroughly by low-speed vortexing until visibly homogeneity. EDTA-plasma specimens were centrifuged at 13

500 and serum specimens at 3 500 relative centrifugal force for 30 minutes, the clear supernatants were then transferred to the sample cups.

Cardiac troponins

Serum cTnI was measured with a high sensitivity assay (STAT High Sensitive Troponin I) on an Architect i2000SR platform (Abbott Diagnostics). The analytic measurement range for this assay is 0-50 000 ng/L, the limit of blank 0.8 ng/L, the lower detection limit 1.2 ng/L, and the coefficient of variation (CV) 10% at a concentration of 3.0 ng/L[12]. Using control material we measured a CV of 4.0% in the low concentration range (20ng/L) and 3.6% in the high concentration range (15 000 ng/L). Concentrations below or equal to the limit of blank were assigned a value of 0.8 ng/L, whereas levels below or equal to the limit of detection and greater than the limit of blank were assigned a value of 1.2 ng/L.

Serum cTnT was measured by a high sensitivity assay (Troponin T hs STAT) on a cobas 8000 e602 analyzer (Roche Diagnostics). The analytic measurement range is 3-10 000 ng/L, limit of blank 3 ng/L, level of detection 5 ng/L, and the CV 10% at a concentration of 13.0 ng/L. Using control material we measured a CV of 3% in the low concentration range (12 pg/mL) and 6 % in the high concentration range (919 pg/mL). Concentrations below or equal to the limit of blank were assigned a value of 3.0 ng/L, whereas levels below or equal to the limit of detection and greater than the limit of blank were assigned a value of 5 ng/L.

Natriuretic peptides

BNP in plasma was analyzed by an ARCHITECT BNP assay on an Architect i2000SR platform (Abbott Diagnostics). The analytical measurement range is 10-5000 pg/mL with a total CV ≤12%. Using control material, we measured a CV of 5.9 % in the low concentration range (90

pg/mL) and 4.8 % in the high concentration range (3500 pg/mL). Samples with levels below 10 pg/mL were assigned a concentration of 5 pg/mL.

NT-proBNP in serum was measured by the proBNP II assay on a cobas 8000 e602 analyzer (Roche Diagnostics). The analytical measurement range is 5 - 35 000 pg/mL with a total CV 2.9-6.1%. Using control material, we measured a CV of 7 % in the low concentration range (99.2 pg/mL) and 6 % in the high concentration range (497.5 pg/mL). The level of detection was 5 pg/mL.

Galectin-3

Galectin-3 in plasma was measured by an ARCHITECT galectin-3 assay on an ARCHITECT i2000SR platform (Abbott Diagnostics). The analytic measurement range is 4.0 -114.0 ng/mL with a total CV ≤ 10%. The limit of blank is 0.8 ng/mL and the level of detection 1.0 ng/mL. Using control material, we measured a CV of 4.6% in the low concentration range (9.1 ng/mL) and 3.3 % in the high concentration range (74.1 ng/mL).

CRP

CRP in serum was measured by a high sensitivity assay (CRP Vario) on an ARCHITECT cSystems platform (Abbott Diagnostics). The analytic measurement range is 0.1-160 mg/L with a total CV ≤ 6%. Using control material, we measured a CV of 3.1% in the low concentration range (1.42 mg/L) and 1.8 % in the high concentration range (23.08 mg/L).

Statistical analysis

Power calculations for the PRADA study were performed for the primary endpoint, i.e. change in LVEF. For this substudy,assuming alpha of 0.05 and an expected correlation coefficient between

0.25 and 0.30, a retrospectively performed power calculation showed that a sample size between 62 and 123 was needed to have a power of 80% to detect an association between change in cardiac troponin and change in LVEF.

Analyses concerning the effect of randomized interventions were conducted according to the intention-to-treat principle. The Shapiro-Wilk test was used to test for normality. Normally distributed continuous data are presented as mean ± standard deviation (SD), non-normally distributed continuous data as median and interquartile range (IQR), and categorical variables as proportions. For normally distributed continuous data paired-sample and independent sample Student`s *t*-tests were used to assess within and between group differences, for non-normally distributed continuous data Wilcoxon Signed Rank and Mann-Whitney U tests were used. Multivariate linear regression was used to assess the relationship between biomarkers and left ventricular function after adjusting for variables that may affect left ventricular function. We did not correct for multiple comparisons, but a hierarchy of biomarkers was prospectively defined in the PRADA statistical analysis plan, which was signed and locked before data unblinding and analysis. Circulating cardiac troponins were defined as secondary end-points and other biomarkers as tertiary end-points. All tests were two-sided and a p-value <0.05 was considered statistically significant. The statistical analyses were carried out with IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY. Retrospective power calculations were performed by using sample size calculators for designing clinical research [13].

Results

Baseline characteristics

All 121 women received the anthracycline epirubicin. In accordance with the national guidelines for adjuvant breast cancer treatment in Norway applicable from September 2011 to November

2015, 27 women were treated with a mean cumulative epirubicin dose of 400 mg/m^2 ± 0 and 94 with doses between 240-360 mg/m^2 (mean cumulative dose of 269 mg/m^2 ± 52). The baseline characteristics are summarized in Table 1. The following biomarkers were evaluated: cTnI, cTnT, BNP, NT-proBNP, galectin-3 and CRP. The number and proportion of patients with detectable levels of troponins are presented in Figure 2.

Cardiotoxicity during anthracycline treatment

In total, 111 patients had LVEF measured by CMR at baseline and at the completion of anthracycline therapy. LVEF declined from 63.3±4.0% to 60.8±4.5% (p=0.005) in the placebo group. One patient who received 400 mg/m^2 of epirubicin fulfilled the criterion for cardiotoxicity as defined by the Cardiac Review and Evaluation Committee Criteria for Cardiotoxicity[14], i.e. LVEF declined from 62.7% to 51.0% without symptoms of heart failure.

Other correlative CMR markers of cardiac injury also increased. There was a dose-dependent increase of pericardial effusion (Table 2), while there was a numerically modest, borderline significant increase in T2 ratio (p=0.053). Late gadolinium enhancement showed no new or increasing areas of focal fibrosis.

Longitudinal change of circulating biomarkers

Longitudinal values and change of the biomarker levels from baseline to completion of anthracycline therapy are summarized in Figure 3. The median levels of cTnI, cTnT, BNP, NT-proBNP, galectin-3 and CRP increased from baseline to completion of anthracycline (all p<0.05) (Table 3). The increases in cTnI, cTnT and CRP concentration were significantly higher in those receiving higher vs. lower doses of anthracycline (all p<0.01), while no clear dose-dependency was observed for the increase in BNP, NT-proBNP and galectin-3 levels (Figure 3).

Effect of metoprolol and candesartan on levels of circulating biomarkers

The effect of metoprolol and candesartan on levels of circulating biomarkers is summarized in Tables 3 and 4. The concentrations of cTnI and cTnT increased less in patients assigned to metoprolol than those not assigned metoprolol. Thus, cTnI increased from 0.8 (0.8, 1.2) to 4.4 (2.5, 7.6) ng/L in patients assigned to metoprolol and from 1.2 (0.8, 1.5) to 7.2 (3.4, 11.8) ng/L in those not assigned to metoprolol (between group difference p=0.019). cTnT increased from 3.0 (3.0, 5.0) to 6.8 (5.0, 10.9) ng/L in patients assigned to metoprolol and from 3.0 (3.0, 5.0) ng/L to 9.7 (6.5, 13.1) ng/L in those not assigned to metoprolol (between group difference p=0.020). The troponin increase in the no metoprolol group was higher in those treated with a cumulative anthracycline dose of 400 mg/m^2 than < 400 mg/m^2 (Table 5). There was no difference between those assigned to candesartan or not. As there was no interaction between the effect of metoprolol and candesartan, the 2x2 factorial design permits comparison of the no metoprolol group with the metoprolol group. Similarly, the no candesartan group can validly be compared with the candesartan group. The validity of this approach is supported by data presented in Tables 6 and 7, showing that there is no significant difference in change in cardiac troponin levels between patients assigned to metoprolol compared to the candesartan-metoprolol combination and those assigned to candesartan compared to the candesartan-metoprolol combination.

The levels of BNP increased from 10.4 (5.0, 21.6) to 15.5 (5.0, 31.5) pg/mL and for NT-proBNP from 52.7 (38.1, 78.2) to 76.8 (41.9, 154.3) pg/mL in patients assigned to metoprolol while concentrations did not change significantly in those not assigned to metoprolol (between group differences for BNP: p = 0.047; for NT-proBNP: p=0.003). There were no between group differences for those assigned to candesartan or not. The interventions did not influence the circulating levels of galecetin-3 or CRP.

Circulating biomarkers and left ventricular systolic and diastolic function

There was no association between on-treatment change in biomarker values and change in left ventricular systolic or diastolic function in multivariate linear regression analysis adjusted for age, body mass index, epirubicin dose, systolic blood pressure, candesartan and metoprolol, (Table 8, 9 and 10). Established cut point values for myocardial infarction for cTnI are levels above 26 ng/L and for cTnT levels above 14ng/L. None of the women had values above these levels at baseline. At completion of anthracycline containing chemotherapy five women had values above the cut point for myocardial infarction for cTnI and 18 for cTnT.

Discussion

The salient findings of the current study of early breast cancer patients are: (i) circulating cTnI, cTnT, BNP, NT-proBNP, galectin-3 and CRP all increase during anthracycline therapy and for cTnI, cTnT and CRP the increase is dose-dependent; (ii) the cTnI and cTnT responses are attenuated by metoprolol, compatible with a beneficial effect on early cardiotoxic injury; (iii) candesartan has no apparent impact on circulating levels of biomarkers of myocardial injury, function, inflammation or fibrosis, and (iv) finally on-treatment change in biomarker concentrations are not associated with early change in left ventricular systolic or diastolic function. These findings provide insight in the effects of beta-adrenergic and angiotensin blockade during anthracycline breast cancer therapy and have important implications for the interpretation and use of cardiovascular biomarkers as monitoring and prognostic tools during adjuvant breast cancer therapy.

Cardiotoxicity during anthracycline treatment

The decline in LVEF in the placebo group in this substudy was 2.5 percentage points. Even though this may be considered a small effect, the magnitude is comparable to findings in other recent studies[15, 16]. Considering that the PRADA study population had a low prevalence of cardiac risk factors and comorbidities and received contemporary anthracycline doses, the observed dose-dependent biomarker changes are likely to be real and reflect a cardiotoxic signal. This early sign of cardiotoxicity is supported by a significant and dose-dependent increase in pericardial effusion and borderline significant increase in T2 ratio.

Longitudinal change of biomarkers

Different classes of cardiovascular biomarkers are thought to provide information concerning different pathophysiological mechanisms[17]. As anthracycline therapy is associated with both cardiomyocyte injury, loss of cardiac contractile function, inflammation and development of diffuse fibrosis[18], we selected biomarkers that are believed to reflect these processes in our study. Cardiac troponins are markers of cardiomyocyte injury and are associated with risk for cardiovascular death and heart failure[19]. Moreover, the use of high sensitivity assays for cTnI and cTnT also permits detection and monitoring of low-grade, chronic myocardial injury[20]. BNP and NT-proBNP are associated with cardiac function and provides strong prognostic information across the spectrum of cardiovascular disease[21-23]. CRP is a prototypical inflammatory biomarker that has been associated with the incidence of cardiovascular disease and death both in the general population, in patients with coronary artery disease and in heart failure[24]. Galectin-3 is a novel biomarker secreted by activated macrophages, thought to reflect myofibroblast proliferation, macrophage migration, inflammation, cardiac remodeling and fibrosis[25-27]. Although some prior studies have reported increase in one or more of all these biomarkers, results have not been consistent[5]. The reasons for these inconsistencies may result from

heterogeneity in patient populations (e.g. different cancer types, cardiovascular disease and /or risk factors), type and dosage of anthracyclines used, timing of blood samples, and the sensitivity of biomarker assays used. For instance, Cardinale et al reported a high proportion of detectable troponin I values measured with a conventional assay with limited sensitivity (lower limit of detection= 350 ng/L) after high-dose anthracycline therapy[6]. More recent but smaller studies using higher sensitivity assays in patients with breast cancer receiving contemporary doses of anthracyclines have also reported an increase of cTnI and CRP during anthracycline treatment but found no change in NT-proBNP and galectin-3[2]. The current study using high sensitivity assays confirms and extends information from prior studies by demonstrating an increase in all biomarkers investigated.

In accordance with earlier findings, these observations suggest that anthracycline therapy at contemporary doses is associated with myocardial injury, inflammation and fibrosis, whereas the increase in biomarkers of cardiac dysfunction such as BNP and NT-proBNP, and reduction in cardiac function evaluated by imaging modalities, seems to be more modest. Moreover, the observation that there was a dose-dependent increase in cTnI, cTnT and CRP suggests that these biomarkers may represent the best tools to monitor the immediate cardiotoxic effects of anthracyclines.

Clearly, the analysis and interpretation of the results may be affected by the kinetics of the different biomarkers. The kinetics of the cardiac troponin, natriuretic peptide, CRP and galectin-3 response following anthracycline treatment have not been clearly defined, but are likely to vary considerably. In our study cardiac troponins were defined as the biomarkers of primary interest. Accordingly, one important consideration for the timing of blood sampling following anthracycline therapy was to be within a time window where cardiac troponin concentrations could be expected to be elevated. As we observed a significant increase in all biomarkers, we

believe the timing of blood sampling was appropriate. To capture the peak level for all biomarkers, daily blood sampling would have been required but this was neither logistically feasible nor ethically acceptable.

Effect of metoprolol and candesartan on levels of circulating biomarkers

The sympathetic nervous system and the renin-angiotensin-aldosterone system exert diverse and complex actions on the myocardium. Blockade of these neuroendocrine systems beneficially modulate the remodeling process that occurs following myocardial injury[28, 29]. In the current substudy candesartan had no effect on the direct cardiotoxic effect of anthracyclines that leads to troponin leakage whereas in the primary analysis of the PRADA study angiotensin blockade with candesartan prevented decline in LVEF that occurred after adjuvant breast cancer therapy with anthracycline with or without radiation and/or trastuzumab[10]. The reason for this apparent discrepancy is likely the beneficial effect of angiotensin blockade on cardiac remodeling[30, 31], which appears to occur independently of the magnitude of cardiotoxic injury, as assessed by cTnI and cTnT measurements. Conversely, the current study suggests that beta-blockade with metoprolol may beneficially impact on the acute toxicity of anthracyclines, reflected in significantly less increase in cTnI and cTnT levels during anthracycline therapy while it had no apparent effect on LVEF in the main analysis. Although the current study is not designed to elucidate the exact mechanisms whereby metoprolol reduces myocardial injury and subsequent cardiac troponin release, a potential mechanism mediating this anti-cardiotoxic effect is the inhibition of beta-adrenergic mediated proapoptotic pathways[32-35]. The clinical significance of our observation is unclear as the increase in cardiac troponin levels was not associated with change in ventricular function from baseline to completion of anthracycline therapy. However, until longer-term follow-up data is available, a cohesive conclusion cannot be drawn concerning

whether the attenuation of troponin increase by metoprolol or the attenuation of decline in LVEF by candesartan is of greater long-term prognostic importance. Based on the information currently available, it may be argued that combined beta-adrenergic and angiotensin blockade represents the reasonable approach for prophylactic cardioprotective therapy in these patients, while definitive conclusion will await data from longer follow up and additional studies.

The observation that metoprolol therapy was associated with higher concentrations of BNP and NT-proBNP was not unexpected, as beta blockers have been shown to increase natriuretic peptide concentrations in healthy subjects as well as in a variety of clinical settings[36]. One potential mechanism is increased stretch of cardiomyocytes induced by the higher end-diastolic volume secondary to the reduction in heart rate by metoprolol. Galectin-3 and CRP were not affected by either of the interventions, suggesting that neuroendocrine blockade with candesartan and metoprolol does not affect the inflammatory and pro-fibrotic response to anthracycline therapy.

Association between individual biomarkers and cardiac function

The literature is inconsistent regarding the association between different cardiac biomarkers and the impairment of cardiac function[5]. In the current study there were no associations between change in biomarkers levels and subsequent change in cardiac systolic and diastolic function during contemporary doses of anthracycline treatment. This suggests that circulating biomarkers have limited potential to predict early reduction in ventricular function; however, we cannot rule out a stronger association in populations with pre-existing cardiovascular disease or with a higher cardiovascular risk factor burden leading to a more pronounced decline in cardiac function. Also there may be a stronger association in patients receiving higher doses of anthracycline or in those reintroduced to anthracyclines because of tumor recurrence. Although the lack of association

between change in biomarkers and change in cardiac function was consistent for the biomarkers examined, we recognize that the relatively modest sample size may have contributed to the lack of association. The question whether an early biomarker response may be predictive of late reduction in ventricular function must await long-term follow-up.

Strengths and limitations

Strengths of the current study include its randomized, 2 x 2 factorial, double blind design, permitting a head-to-head comparison of two different drugs. Also, the study population was well characterized phenotypically and homogeneous with little comorbidity. Importantly, the anthracycline doses used in this study were in accordance with contemporary guidelines for breast cancer treatment. Limitations of the current report include the lack of follow-up information beyond the adjuvant treatment period, but long-term follow-up is planned and ongoing. Also, the kinetic profiles of the different biomarkers during and after anthracycline therapy have not been clearly defined, and the optimal timing for biomarker sampling could have been missed.

Conclusions

In patients receiving contemporary treatment for early breast cancer cTnI, cTnT, and CRP increased during chemotherapy in a dose dependent fashion. Long-term patient follow-up is required to determine whether the impact of metoprolol on cardiac troponin levels during therapy will translate into clinical benefit. Likewise, the lack of associations between change in biomarker concentrations and early changes in ventricular function suggest that the clinical utility of these biomarkers as prognostic tools is limited, but long-term studies are warranted.

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Figure legends:

Figure 1:

The timing of blood sampling and cardiac imaging

The time of the first FEC cycle defines day 0. Blood sampling, cardiac imaging and last FEC cycle are shown in relation to the first FEC cycle. Values are given as median (IQR). IQR interquartile range; CMR cardiovascular magnetic resonance; Echo echocardiography, FEC 5-fluorouracil, epirubicin, cyclophosphamide;

Figure 2:

Distribution of cardiac troponin T and I concentrations before and after anthracycline treatment.

Blue bars represent values below or equal to the level of detection and green bars values greater than the level of detection.

cTn cardiac troponin

Figure 3:

Change in median values of biomarker from baseline to completion of anthracycline therapy

Median biomarker values at baseline and at completion of anthracycline therapy for those treated with a total mean cumulative epirubicin dose of 400 mg/m^2 (in orange) and <400 mg/m^2 (in grey). Bars represent the interquartile range, * represents significant increase ($p<0.05$) of the biomarker from baseline to end of anthracycline containing chemotherapy. P-value is for median between group differences.

cTn cardiac troponin; BNP B-type natriuretic peptides; NT-proBNP Amino-terminal fragment of the BNP prohormone; CRP C-reactive protein**;** Epi epirubicin

Table 1: Baseline characteristics

	Epirubicin = 400 mg/m^2 (n =27)		Epirubicin < 400 mg/m^2 (n= 94)	
Age (year)	51.0	(42.0, 59.0)	48.5	(43.8, 58.0)
Body mass index	24.5	(22.2, 27.2)	25.9	(22.9, 29.1)
Systolic blood pressure (mmHg)	135.0	(120.0, 140.0)	128.5	(120.0, 140.0)
Diastolic blood pressure (mmHg)	80.0	(75.0, 85.0)	80.0	(74.5, 85.0)
Heart rate (beats/minute)	71.5	±10.3	70.9	±10.0
Hypertension (n)	1	(7%)	7	(7.4%)
Diabetes Mellitus (n)	0	(0%)	2	(2.1%)
Current Smoking (n)	6	(22.2%)	19	(20.2%)
Serum creatinine mg/dl	0.75	(0.69, 0.81)	0.72	(0.69, 0.81)
Hemoglobin g/dl	13.2	±0.94	13.3	±0.81
FEC treatment				
FEC 60 mg/m^2 x 4 (n)	0		71 (75.5%)	
FEC 60 mg/m^2 x 6 (n)	0		23 (24.5%)	
FEC 100 mg/m^2 x 4 (n)	27 (100%)		0	
HER2 status	positive		Negative	

Data are expressed as mean ± SD, median (interquartile range) or numbers (percent); FEC 5-fluorouracil, epirubicin, cyclophosphamide; HER human epidermal growth factor receptor; SD standard deviation

Table 2: Cardiovascular magnetic resonance markers of cardiac injury in the whole population and in those assigned to a cumulative anthracycline dose of < 400 mg/m2 and equal to 400 mg/m2

	n	Baseline	After anthracycline*	p-value	Between group p-value
Pericardial effusion (mm)					
All	111	1 (0, 3)	2 (0, 4)	0.003	
< 400 mg/m^2	87	1 (0, 3)	2 (0, 3)	0.175	0.001
= 400 mg/m^2	24	1 (0, 2)	3 (2, 4)	0.001	
T2 (ratio)**					
All	109	1.86 ±0.24	1.91 ±0.23	0.053	
< 400 mg/m^2	85	1.85 ±0.23	1.91 ±0.23	0.101	0.953
= 400 mg/m^2	24	1.89 ±0.28	1.95 ±0.24	0.311	

*anthracycline containing chemotherapy with 5-fluorouracil, epirubicin, cyclophosphamide (FEC); Values are given in median (IQR) for non-normally distributed data, Wilcoxon Signed Rank and Mann-Whitney U tests are used. Values are given in mean ±SD for normally distributed data and Student`s *t*-tests were used. **The T2 ratio is between the T2 signal intensity in myocardium and skeletal muscle; IQR interquartile range; SD standard deviation; n numbers; ms milliseconds

Table 3: Comparison of change in biomarkers in all patients and in those assigned to and not assigned to metoprolol

	n	Baseline median values (IQR)	After anthracycline* median values (IQR)	Median Difference (IQR)	Within group p-value	Between group p-value
cTnI ng/L						
All	121	0.8 (0.8, 1.4)	5.6 (3.0, 9.3)	4.4 (1.8, 7.8)	<0.001	
Metoprolol	62	0.8 (0.8, 1.2)	4.4 (2.5, 7.6)	2.9 (1.6, 6.8)	<0.001	0.019
No Metoprolol	59	1.2 (0.8, 1.5)	7.2 (3.4, 11.8)	5.7 (2.3, 10.0)	<0.001	
cTnT ng/L						
All	121	3.0 (3.0, 5.0)	8.5 (5.6, 12.7)	4.3 (2.0, 8.0)	<0.001	
Metoprolol	62	3.0 (3.0, 5.0)	6.8 (5.0, 10.9)	3.4 (2.0, 7.4)	<0.001	0.020
No Metoprolol	59	3.0 (3.0, 5.0)	9.7 (6.5, 13.1)	5.0 (2.6, 9.9)	<0.001	
BNP pg/ml						
All	119	10.4 (5.0, 19.1)	12.0 (5.0, 23.0)	0.0 (-3.2, 10.5)	0.049	
Metoprolol	62	10.4 (5.0, 21.6)	15.5 (5.0, 31.5)	0.4 (-2.0, 13.6)	0.005	0.047
No Metoprolol	57	10.5 (5.0, 16.3)	5.0 (5.0, 18.1)	0.0 (-5.9, 2.5)	0.882	
NT-proBNP pg/ml						
All	121	48.3 (32.0, 76.5)	55.2 (29.5, 98.1)	10 (-13.1, 41.2)	0.002	
Metoprolol	62	52.7 (38.1, 78.2)	76.8 (41.9, 154.3)	17.7 (2.0, 59.5)	<0.001	0.003
No Metoprolol	59	42.7 (29.1, 74.1)	49.2 (23.0, 68.1)	1.2 (-22.8, 25.6)	0.721	
Galectin-3 ng/mL						
All	120	12.1 (10.4, 14.0)	13.4 (11.2, 16.0)	1.1 (0.0, 2.4)	<0.001	
Metoprolol	62	11.9 (10.1, 13.9)	13.5 (11.5, 16.3)	1.7 (-0.0. 2.9)	<0.001	0.119
No Metoprolol	58	12.2 (10.6, 14.2)	13.3 (11.2, 15.7)	0.9 (-0.0, 2.0)	<0.001	
CRP mg/L						
All	121	1.9 (0.9, 4.3)	2.9 (1.2, 6.5)	0.3 (-0.9, 2.7)	0.019	
Metoprolol	62	1.9 (0.9, 5.0)	2.1 (1.0, 6.4)	0.3 (-0.8, 2.3)	0.081	0.979
No Metoprolol	59	2.0 (1.0, 4.3)	3.3 (1.3, 6.8)	0.3 (-1.3, 3.6)	0.111	

*anthracycline containing chemotherapy with 5-fluorouracil, epirubicin, cyclophosphamide (FEC); IQR inter quartile range; cTn cardiac troponin; BNP B-type natriuretic peptides; NT-proBNP Amino-terminal fragment of the BNP prohormone; CRP C-reactive protein

Table 4: Comparison of change in biomarkers in patients assigned to and not assigned to candesartan

	n	Baseline median values (IQR)	After anthracycline* median values (IQR)	Median Difference (IQR)	Within group p-value	Between group p-value
cTnI ng/L						
Candesartan	62	0.8 (0.8, 1.4)	5.6 (3.0, 9.1)	4.2 (1.9, 7.3)	<0.001	0.846
No Candesartan	59	1.2 (0.8, 1.4)	5.6 (2.9, 9.9)	4.8 (1.7, 9.1)	<0.001	
cTnT ng/L						
Candesartan	62	3.0 (3.0, 5.0)	8.7 (5.8, 12.6)	4.1 (2.0, 8.3)	<0.001	0.942
No Candesartan	59	3.0 (3.0, 5.0)	8.0 (5.6, 13.0)	4.5 (2.0, 7.9)	<0.001	
BNP pg/ml						
Candesartan	62	5.0 (5.0, 20.8)	11.8 (5.0, 20.2)	0.0 (-3.8, 12.9)	0.048	0.453
No Candesartan	57	11.9 (5.0, 18.8)	12.3 (5.0, 23.8)	0.0 (-3.5, 8.0)	0.401	
NT-proBNP pg/ml						
Candesartan	62	48.6 (30.5, 77.8)	53.3 (27.0, 93.5)	9.5 (-11.8, 33.9)	0.054	0.717
No Candesartan	59	48.2 (35.7, 75.4)	58.9 (31.3, 99.2)	10.1 (-15.9, 45.3)	0.022	
Galectin-3 ng/mL						
Candesartan	62	11.9 (9.9, 14.3)	13.2 (11.0, 16.2)	0.9 (-0.2, 2.4)	<0.001	0.414
No Candesartan	58	12.2 (10.7, 13.9)	13.5 (11.9, 15.8)	1.6 (0.2, 2.4)	<0.001	
CRP mg/L						
Candesartan	62	1.8 (1.0, 3.7)	2.3 (0.9, 6.4)	0.2 (-0.8, 2.6)	0.203	0.454
No Candesartan	59	1.9 (0.9, 5.3)	3.2 (1.5, 8.0)	0.8 (-1.4, 3.7)	0.060	

*anthracycline containing chemotherapy with 5-fluorouracil, epirubicin, cyclophosphamide (FEC); IQR inter quartile range; cTn cardiac troponin; BNP B-type natriuretic peptides; NT-proBNP Amino-terminal fragment of the BNP prohormone; CRP C-reactive protein

Table 5: Cardiac troponin I and T concentrations according to the cumulative anthracycline dose in the whole cohort and stratified for beta blockade

	n	Median Δ value for cumulative anthracycline* dose <400 mg/m^2 (IQR)	n	Median Δ value for cumulative anthracycline* dose = 400 mg/m^2 (IQR)	p-value
cTnI					
All	94	3.8 (1.6, 6.9)	27	6.4 (2.5, 13.5)	0.009
Metoprolol	46	2.8 (1.4, 6.3)	13	4.3 (2.4, 11.6)	0.094
No metoprolol	48	5.1 (1.9, 8.9)	14	9.6 (4.7, 15.1)	0.036
cTnT					
All	94	3.6 (2.0, 7.4)	27	7.5 (3.6, 11.7)	0.002
Metoprolol	46	2.7 (1.5, 7.1)	13	5.8 (3.0, 10.3)	0.031
No metoprolol	48	4.5 (2.3, 7.7)	14	8.7 (4.9, 13.6)	0.019

*anthracycline containing chemotherapy in combination with 5-fluorouracil and cyclophosphamide. cTn cardiac troponin; IQR interquartile range

Table 6: Comparison of cardiac troponin levels in patients assigned to metoprolol only and those assigned to metoprolol and candesartan

		n	Baseline median values (IQR)	After anthracycline* median values (IQR)	Median difference (IQR)	Between group p-value
cTnI	Metoprolol only	29	0.8 (0.8, 1.3)	4.7 (2.2, 8.1)	2.7 (1.4, 7.1)	0.96
	Candesartan and metoprolol	30	0.8 (0.8, 1.2)	4.4 (2.8, 7.5)	3.2 (1.7, 6.4)	
cTnT	Metoprolol only	29	3.0 (3.0, 5.0)	6.8 (5.0, 12.0)	3.0 (1.9, 7.7)	0.80
	Candesartan and metoprolol	30	3.0 (3.0, 5.0)	6.8 (5.0, 10.5)	3.6 (2.0, 7.2)	

*anthracycline containing chemotherapy in combination with 5-fluorouracil and cyclophosphamide. cTn cardiac troponin; IQR interquartile range

Table 7: Comparison of cardiac troponin levels in patients assigned to candesartan only and those assigned to metoprolol and candesartan

		n	Baseline median values (IQR)	After anthracycline* median values (IQR)	Median difference (IQR)	Between group p-value
cTnI	Candesartan only	32	0.8 (0.8, 1.5)	7.3 (3.4,12.2)	5.7 (2.2, 10.3)	0.08
	Candesartan and metoprolol	30	0.8 (0.8, 1.2)	4.4 (2.8,7.5)	3.2 (1.7, 6.4)	
cTnT	Candesartan only	32	3.0 (3.0, 5.0)	10.2 (6.6,14.0)	5.2 (2.1, 10.0)	0.13
	Candesartan and metoprolol	30	3.0 (3.0, 5.0)	6.8 (5.0,10.5)	3.6 (2.0, 7.2)	

*anthracycline containing chemotherapy in combination with 5-fluorouracil and cyclophosphamide. cTn cardiac troponin; IQR interquartile range

Table 8: Multivariate linear regression for assessing association between change in circulating cardiovascular biomarkers and change in left ventricular ejection fraction (LVEF) as measured by cardiovascular magnetic resonance imaging with adjustment for variables that could affect change in LVEF

Variables	B*	95% confidence interval for B	p value	Variables	B*	95% confidence interval for B	p value
ΔcTnI	-0.04	(-0.13, 0.05)	0.340	ΔcTnT	-0.09	(-0.24, 0.05)	0.198
Age (years)	0.04	(-0.05, 0.14)	0.381	Age (years)	0.05	(-0.04, 0.15)	0.283
BMI (kg/m^2)	0.11	(-0.08, 0.30)	0.257	BMI (kg/m^2)	0.11	(-0.08, 0.29)	0.257
SBP (mmHg)	-0.01	(-0.08, 0.06)	0.872	SBP	-0.01	(-0.08, 0.06)	0.848
Epi. dose†	-1.48	(-3.47, 0.52)	0.146	Epi. dose†	-1.29	(-3.32, 0.75)	0.213
Candesartan	1.44	(-0.18, 3.07)	0.081	Candesartan	1.50	(-0.11, 3.12)	0.068
Metoprolol	0.67	(-0.97, 2.31)	0.419	Metoprolol	0.65	(-0.97, 2.27)	0.428
ΔBNP	0.01	(-0.04, 0.06)	0.584	ΔNT-proBNP	0.00	(-0.01, 0.02)	0.875
Age (years)	0.03	(-0.07, 0.13)	0.531	Age (years)	0.04	(-0.06, 0.13)	0.436
BMI (kg/m^2)	0.11	(-0.08, 0.30)	0.257	BMI (kg/m^2)	0.10	(-0.09, 0.29)	0.304
SBP (mmHg)	-0.01	(-0.08, 0.06)	0.759	SBP (mmHg)	-0.01	(-0.08, 0.06)	0.865
Epi. dose†	-1.77	(-3.79, 0.26)	0.087	Epi. dose†	-1.64	(-3.65, 0.36)	0.107
Candesartan	1.63	(-0.02, 3.29)	0.053	Candesartan	1.49	(-0.14, 3.12)	0.073
Metoprolol	0.55	(-1.12, 2.23)	0.513	Metoprolol	0.80	(-0.91, 2.51)	0.356
ΔGalectin-3	0.12	(-0.23, 0.46)	0.499	ΔCRP	-0.05	(-0.11, 0.01)	0.120
Age (years)	0.03	(-0.07, 0.13)	0.530	Age (years)	0.04	(-0.05, 0.13)	0.389
BMI (kg/m^2)	0.11	(-0.08, 0.30)	0.242	BMI (kg/m^2)	0.08	(-0.10, 0.27)	0.386
SBP (mmHg)	-0.01	(-0.08, 0.06)	0.855	SBP (mmHg)	-0.01	(-0.08, 0.06)	0.877
Epi. dose†	-1.63	(-3.63, 0.37)	0.109	Epi. dose†	-1.35	(-3.34, 0.64)	0.180
Candesartan	1.65	(0.01, 3.29)	0.049	Candesartan	1.53	(-0.08, 3.14)	0.062
Metoprolol	0.63	(-1.02, 2.28)	0.452	Metoprolol	0.94	(-0.65, 2.53)	0.244

*B unstandardized regression coefficient; †dichotomized variable for cumulative epirubicin dose of 400mg/m^2 and <400mg/m^2; cTnI cardiac troponin I; BMI body mass index; SBP systolic blood pressure at baseline; cTnT cardiac troponin T; BNP B-type natriuretic peptides; NT-proBNP Amino-terminal fragment of the BNP prohormone; CRP C-reactive protein

Table 9: Multivariate linear regression for assessing association between change in circulating cardiovascular biomarkers and change in peak systolic global longitudinal strain (GLS) by echocardiography with adjustment for variables that could affect change in GLS

Variables	B*	95% confidence interval for B	p value	Variables	B*	95% confidence interval for B	p value
ΔcTnI	-0.02	(-0.07, 0.02)	0.303	ΔcTnT	-0.03	(-0.11, 0.04)	0.387
Age (years)	-0.04	(-0.09, 0.01)	0.121	Age (years)	-0.04	(-0.09, 0.02)	0.162
BMI (kg/m^2)	-0.08	(-0.18, 0.02)	0.115	BMI (kg/m^2)	-0.09	(-0.19, 0.01)	0.087
SBP (mmHg)	-0.03	(-0.06, 0.01)	0.111	SBP (mmHg)	-0.03	(-0.06, 0.01)	0.120
Epi. dose†	-0.06	(-1.09, 0.96)	0.904	Epi. dose†	0.01	(-1.05, 1.05)	0.993
Candesartan	-0.07	(-0.92, 0.77)	0.862	Candesartan	-0.05	(-0.89, 0.79)	0.910
Metoprolol	-0.08	(-0.93, 0.76)	0.845	Metoprolol	-0.05	(-0.88, 0.79)	0.914
ΔBNP[l]	0.02	(-0.01, 0.04)	0.180	ΔNT-proBNP	0.01	(0.00, 0.01)	0.169
Age (years)	-0.04	(-0.09, 0.01)	0.106	Age (years)	-0.04	(-0.09, 0.01)	0.083
BMI (kg/m^2)	-0.07	(-0.18, 0.03)	0.152	BMI (kg/m^2)	-0.08	(-0.18, 0.02)	0.126
SBP (mmHg)	-0.04	(-0.07, 0.00)	0.061	SBP (mmHg)	-0.03	(-0.07, 0.00)	0.073
Epi. dose†	-0.05	(-1.07, 0.97)	0.929	Epi. dose†	-0.14	(-1.16, 0.88)	0.783
Candesartan	0.01	(-0.82, 0.84)	0.978	Candesartan	-0.02	(-0.84, 0.80)	0.961
Metoprolol	-0.20	(-1.06, 0.66)	0.645	Metoprolol	-0.17	(-1.02, 0.69)	0.704
ΔGalectin-3	0.04	(-0.19, 0.27)	0.726	ΔCRP	0.00	(-0.03, 0.03)	0.993
Age (years)	-0.04	(-0.09, 0.01)	0.102	Age (years)	-0.04	(-0.09, 0.01)	0.122
BMI (kg/m^2)	-0.08	(-0.18, 0.02)	0.124	BMI (kg/m^2)	-0.09	(-0.19, 0.01)	0.084
SBP (mmHg)	-0.03	(-0.07, 0.01)	0.108	SBP (mmHg)	-0.03	(-0.07, 0.01)	0.114
Epi. dose†	-0.09	(-1.12, 0.94)	0.864	Epi. dose†	-0.11	(-1.16, 0.94)	0.838
Candesartan	0.07	(-0.78, 0.91)	0.875	Candesartan	0.01	(-0.83, 0.84)	0.988
Metoprolol	-0.07	(-0.92, 0.78)	0.872	Metoprolol	0.01	(-0.82, 0.84)	0.975

*B unstandardized regression coefficient; †dichotomized variable for cumulative epirubicin dose of 400mg/m^2 and <400mg/m^2; cTnI cardiac troponin I; BMI body mass index; SBP systolic blood pressure at baseline; cTnT cardiac troponin T; BNP B-type natriuretic peptides; NT-proBNP Amino-terminal fragment of the BNP prohormone; CRP C-reactive protein

Table 10: Multivariate linear regression for assessing association between change in circulating cardiovascular biomarkers and change in the ratio of peak early (E) transmitral velocity by pulsed Doppler and peak early tissue Doppler (E`) (E/E`) by echocardiography with adjustment for variables that could affect change in E/E`

Variables	B*	95% confidence interval for B	p value	Variables	B*	95% confidence interval for B	p value
ΔcTnI	-0.02	(-0.05, 0.02)	0.297	ΔcTnT	-0.04	(-0.09, 0.01)	0.097
Age (years)	-0.02	(-0.06, 0.01)	0.197	Age (years)	-0.02	(-0.06, 0.02)	0.269
BMI (kg/m^2)	-0.03	(-0.09, 0.04)	0.451	BMI (kg/m^2)	-0.02	(-0.09, 0.04)	0.495
SBP (mmHg)	0.02	(-0.01, 0.05)	0.128	SBP (mmHg)	0.02	(-0.01, 0.05)	0.117
Epi. dose†	0.65	(-0.08, 1.37)	0.078	Epi. dose†	0.78	(0.03, 1.52)	0.041
Candesartan	-0.25	(-0.86, 0.35)	0.408	Candesartan	-0.24	(-0.84, 0.36)	0.423
Metoprolol	0.57	(-0.04, 1.18)	0.065	Metoprolol	0.55	(-0.05, 1.16)	0.072
ΔBNP	0.00	(-0.02, 0.01)	0.664	ΔNT-proBNP	0.00	(-0.01, 0.00)	0.286
Age (years)	-0.02	(-0.06, 0.02)	0.259	Age (years)	-0.02	(-0.06, 0.01)	0.223
BMI (kg/m^2)	-0.03	(-0.10, 0.03)	0.328	BMI (kg/m^2)	-0.03	(-0.10, 0.03)	0.296
SBP (mmHg)	0.02	(-0.01, 0.05)	0.124	SBP (mmHg)	0.02	(0.00, 0.05)	0.102
Epi. dose†	0.64	(-0.09, 1.38)	0.085	Epi. dose†	0.65	(-0.08, 1.37)	0.078
Candesartan	-0.28	(-0.89, 0.33)	0.369	Candesartan	-0.24	(-0.84, 0.37)	0.437
Metoprolol	0.73	(0.09, 1.37)	0.025	Metoprolol	0.74	(0.11, 1.37)	0.023
ΔGalectin-3	-0.03	(-0.16, 0.10)	0.673	ΔCRP	0.00	(-0.02, 0.03)	0.698
Age (years)	-0.02	(-0.06, 0.02)	0.240	Age (years)	-0.03	(-0.06, 0.01)	0.187
BMI (kg/m^2)	-0.03	(-0.10, 0.03)	0.330	BMI (kg/m^2)	-0.03	(-0.09, 0.04)	0.392
SBP (mmHg)	0.02	(-0.01, 0.05)	0.144	SBP (mmHg)	0.02	(-0.01, 0.05)	0.148
Epi. dose†	0.60	(-0.13, 1.33)	0.108	Epi. dose†	0.57	(-0.17, 1.30)	0.129
Candesartan	-0.27	(-0.89, 0.34)	0.384	Candesartan	-0.23	(-0.84, 0.37)	0.451
Metoprolol	0.69	(0.06, 1.31)	0.031	Metoprolol	0.62	(0.02, 1.23)	0.044

*B unstandardized regression coefficient; †dichotomized variable for cumulative epirubicin dose of 400mg/m^2 and <400mg/m^2; cTnI cardiac troponin I; BMI body mass index; SBP systolic blood pressure at baseline; cTnT cardiac troponin T; BNP B-type natriuretic peptides; NT-proBNP Amino-terminal fragment of the BNP prohormone; CRP C-reactive protein

CLINICAL PERSPECTIVE

What Is New?

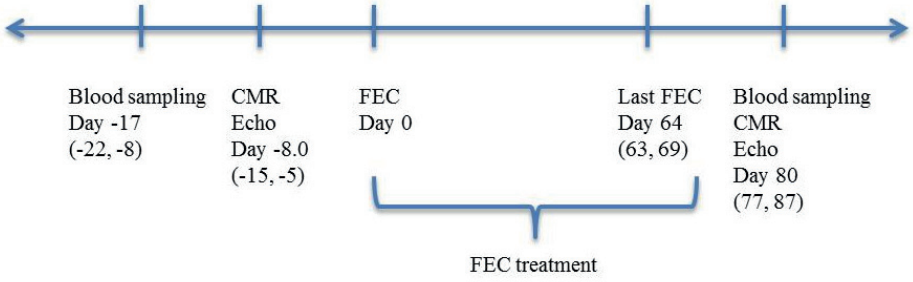
- Treatment with contemporary doses of anthracycline in early breast cancer is associated with increased cardiovascular biomarker concentrations reflecting myocardial injury (cardiac troponins), dysfunction (natriuretic peptides), inflammation (C-reactive protein) and fibrosis (galectin-3).
- Early changes in levels of circulating biomarkers are not diagnostic of early impairment of left ventricular systolic or diastolic function.
- Beta-adrenergic blockade with metoprolol attenuates anthracycline-induced myocardial injury as expressed by increase of circulating troponin concentrations.

What Are the Clinical Implications?

- Preventive beta-adrenergic blockade may have beneficial early effects on anthracycline-induced myocardial injury, but longer-term follow-up will be necessary to evaluate whether this early attenuation of troponins by metoprolol translates into reduced incidence of late cardiotoxicity.

Angiotensin and beta-adrenergic blockade may provide complementary cardio-protective effects during anthracycline therapy

Figure 1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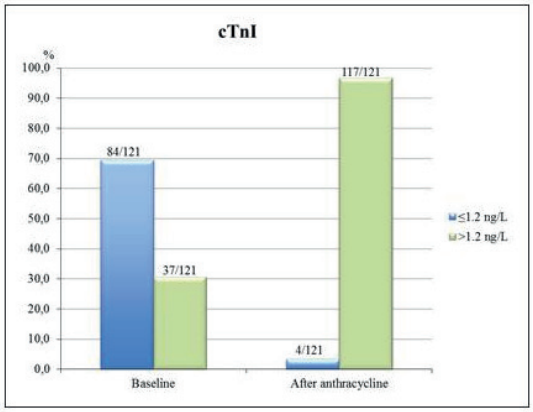
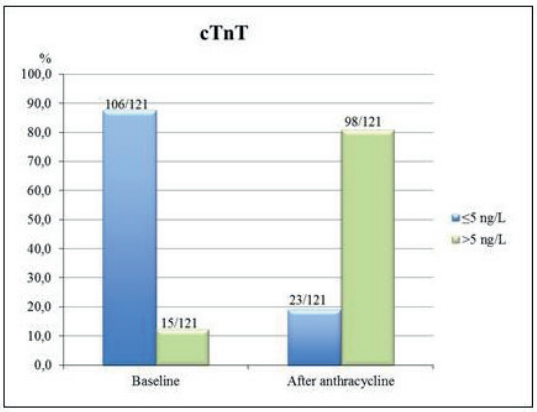
CMR
Echo
Day -8.0
(-15, -5)
FEC
Day 0
Last FEC
Day 64
(63, 69)
Blood sampling
CMR
Echo
Day 80
(77, 87)
FEC treatment

Figure 2


cTnI
%
100,0
90,0
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
84/121
37/121
4/121
117/121
Baseline
After anthracycline
≤1.2 ng/L
>1.2 ng/L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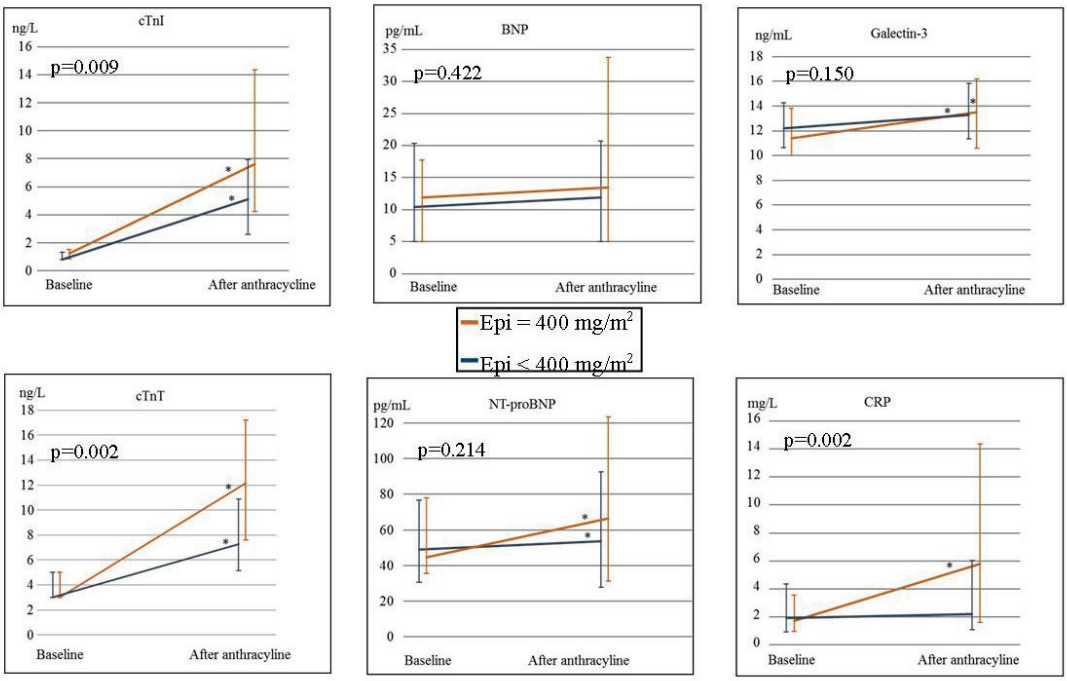
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
106/121
15/121
23/121
98/121
Baseline
After anthracycline
≤5 ng/L
>5 ng/L

Figure 3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ng/L
p=0.009
Baseline
After anthracycline
BNP
pg/mL
p=0.422
Baseline
After anthracyline
Galectin-3
ng/mL
p=0.150
Baseline
After anthracyline
Epi = 400 mg/m²
Epi < 400 mg/m²
cTnT
ng/L
p=0.002
Baseline
After anthracyline
NT-proBNP
pg/mL
p=0.214
Baseline
After anthracyline
CRP
mg/L
p=0.002
Baseline
After anthracyline